Novel Macrocycles and Uses Thereof

Government Support

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Background of the Invention

Many natural products, usually bacterial metabolites, feature a macrolide fused to a monocyclic benzenoid matrix, bearing a resorcinol-like substitution pattern. Not infrequently, the resorcinol moiety carries additional functionality, resulting in higher levels of oxidation. Natural products in this family (cf. inter alia radicicol (Delmotte, P.; Delmotte-Plaquee, J. *Nature* **1953**, 171, 344; incorporated herein by reference), LL-Z-1640s (McGahren, W. J. J. Org. Chem. **1978**, 43, 2339-2343; which is incorporated herein by reference), monocillins (Ayer, W. A.; Lee, S. P.; Tsuneda, A.; Hiratsuka, Y. *Can. J. Microb.* **1980**, 26, 766-773; incorporated herein by reference), nordinone (Ayer, W. A.; Pena-Rodriguez, L. *Phytochemistry* **1987**, 26, 1353-1355; incorporated herein by reference) and zearelenone (Sugawara, F.; Kim, K. W.; Kobayashi, K.; Uzawa, J.; Yoshida, S.; Murofushi, N.; Takahashi, N.; Strobel, G. A. *Phytochemistry* **1992**, 31, 1987-1990; incorporated herein by reference) possess potentially exploitable patterns of antitumor, antibiotic, and antimalarial activity.

Radicicol (Delmotte *et al. Nature* **1953**, *171*, 344; Ayer *et al. Canad. J. Microbiol.* **1980**, *26*, 766; incorporated herein by reference) (**1**) and monocillin I (Ayer *et al. Canad. J. Microbiol.* **1980**, *26*, 766) (**2**) are resorcylic macrolides which can both be isolated from Monocillium nordinii (Ayer *et al. Canad. J. Microbiol.* **1980**, *26*, 766; incorporated herein by reference) (Figure 1). While the skeletal structure of radicicol was determined in 1964, (McCapra *et al. Tetrahedron Lett.* **1964**, 869; Mirrington *et al. Tetrahedron Lett.* **1964**, 365; incorporated herein by reference) its relative and absolute stereochemical configuration was not unambiguously established until 1987 (Cutler *et al. Agric. Biol. Chem.* **1987**, *51*, 3331; incorporated herein by reference). The structure

of monocillin I was confirmed by its direct conversion into radicicol. Affirmation of these structures was achieved by their only total synthesis through the efforts of Lett and Lampilas (Lampilas *et al. Tetrahedron Lett.* **1992**, *33*, 773 and 777; incorporated herein by reference).

Both radicicol (**1**) and monocillin I (**2**) (*see* Figure 1) exhibit a variety of antifungal and antibiotic properties not shared by other members of this class of natural products. Recently, the antitumor properties of radicicol have come into focus as its ability to suppress the transformed phenotype caused by various oncogenes such as *src, ras,* and *raf* has been linked to its tight binding (20 nM) and inhibition of the Hsp90 molecular chaperone (Roe *et al. J. Med. Chem.* **1999**, *42*, 260-266; incorporated herein by reference). This 'anti-chaperone' activity may stimulate depletion of oncogenic proteins, and could therefore be of clinical interest. Specifically, occupancy of the ATP binding pocket of Hsp90 is believed to lead to the degradation in the proteasome of a subset of proteins involved in signal transduction that require Hsp90 for conformational maturation (*see*, Schneider *et al. Proc. Natl. Acad. Sci.* USA *93*: 14536-14541, **1996**; Mimnaugh *et al. J. Biol. Chem. 271*: 22796-22801, **1996**; Whitesell *et al. Mol. Endocrinol. 10*: 705-712, **1996**; each of which is incorporated herein by reference). These proteins include the HER and insulin receptor families of tyrosine kinases, Raf-1 serine kinase and steroid receptors to name a few. Downregulation of any of these would be expected to have positive antiproliferative effects, so that Hsp90 is an attractive target for the development of antitumor drugs.

More recently, five new 14-membered resorcyclic macrolides, termed aigailomycins A-E, were isolated from the marine mangrove fungus *Aigialus parvus* BCC5311 (Isaka, M.; Suyarnsestakorn, C.; Tanticharoen, M.; Kongsaeree, P.; Thebtaranonth, Y. *J. Org. Chem.* **2002**, *67*, 1561-1566; incorporated herein by reference). Among the aigailomycins, aigailomycin D exhibits potent antimalarial activity (IC_{50}: 6.6 μg/mL against *P. falciparum*) and antitumor activity (IC_{50}: 3.0 μg/mL against KB cells) (Isaka, M.; Suyarnsestakorn, C.; Tanticharoen, M.; Kongsaeree, P.; Thebtaranonth, Y. *J. Org. Chem.* **2002**, *67*, 1561-1566; incorporated herein by reference).

The demonstrated ability of radicicol to bind to and inhibit the activity of Hsp90 has generated an interest in further exploring the biological and pharmacological

activity of radicicol and analogues thereof. Significantly, to date, only one synthesis of radicicol itself has been recorded (Lampilas *et al. Tetrahedron Lett.* **1992**, *33*, 773 and 777; incorporated herein by reference). Other groups have accessed a variety of analogues from the natural product itself (see, US Patent 5,650,430; US Patent 5,731,343; US Patent 6,239,168; US Patent 5,977,165; and US Patent 5,597,846; each of which is incorporated herein by reference), but have been limited in the range of analogues that can be generated. Thus, there remains a need to develop a practical synthesis of radicicol and other resorcyclic macrolides to generate novel analogs and conjugates to explore novel biological and pharmacological activities, and to improve the stability and therapeutic efficacy of radicicol, monocillin, and aigialomycins in the treatment of cancer.

DESCRIPTION OF THE DRAWING

Figure 1 depicts structures of Monocillin I **(2)**, Radicicol **(1)** and Geldanamycin **(3)**.

Figure 2 depicts two strategies for the synthesis of radicicol **(1)** and monocillin **(2)**.

Figure 3 depicts the synthetic strategy for the construction of the chiral allylic alcohol.

Figure 4 depicts the synthetic strategy for the construction of intermediate **(18)**.

Figure 5 depicts the synthetic strategy for the construction of intermediate **(7)** via a Mitsunobou esterification.

Figure 6 depicts a synthetic strategy for the synthesis of radicicol **(1)** and monocillin **(2)**.

Figure 7 depicts the synthesis of a variety of chiral components **(28)**, **(30)** and **(32)**.

Figure 8 depicts the synthesis of dithiane fragment **(34)**.

Figure 9 depicts the synthesis of a variety of benzoic acid components **(35)**, **(36)**, **(37)** and **(38)**.

Figure 10 depicts the generation of diversity at aromatic positions in the macrocycle.

Figure 11 depicts the synthesis of a variety of analogues **(40)**, **(42)**, **(44)** and **(46)**.

Figure 12 depicts the synthesis of a variety of analogues **(48)**, **(50)**, **(52)** and **(54)**.

Figure 13 depicts the synthesis of the chiral cyclopropyl moiety **(30)** and generation of intermediate **(39)**.

Figure 14 depicts the synthetic scheme for cyclopropyl-monocillin I **(2c)** and cyclopropyl-radicicol **(40)**.

Figure 15 depicts the synthesis of a variety of inventive conjugates.

Figure 16 depicts the synthesis of a variety of inventive conjugates.

Figure 17 depicts the results of MCF7 cells (HER2 overexpressed, Rb positive) treated with radicicol and analogues.

Figure 18 depicts the results of BT474 cells (HER2 overexpressed, Rb positive) treated with radicicol and analogues. The gels demonstrate reduction of HER2 levels over a range of concentrations.

Figure 19 depicts the growth of MCF7 cells (HER2 overexpressed, Rb positive) treated with radicicol and analogues.

Figure 20 depicts the ability of radicicol and cyclopropyl radicicol to inhibit breast cancer cells with wild type Rb and small cell lung cancer cells with defective Rb function.

Figure 21 depicts the growth curve for N417 cells (Rb negative cell line) for radicicol and analogues thereof.

Figure 22 shows the effect of cycloproparadicicol in nude mice bearing human mammary carcinoma MX-1 xenograft (Q2Dx7, iv injection).

Figure 23 shows the effect of cycloproparadicicol treatment (Q2Dx6, iv injection) on body weight of nude mice bearing mammary carcinoma MX-1 xenograft.

Figure 24 shows the therapeutical effect of radicicol and cycloproparadicicol in nude mice bearing human colon carcinoma (HCT-116) xenograft (QDx7, 4 hr. iv-infusion).

Figure 25 shows body weight changes of nude mice bearing human colon carcinoma (HCT-116) xenografts following treatment with radicicol and cycloproparadicicol (QDx7, 4 hr. iv-infusion).

Figure 26 shows the effect of cycloproparadicicol on HER2 degradation.

Figure 27 lists the IC_{50}s of various radidicicol analogues on growth inhibition in different tumor cell lines.

Figure 28 shows the effect of four different cycloproparadicicol analogues on the degradation of HER2

Figure 29 depicts the structure of difluoro-cycloproparadicicol.

Figure 30 is a table showing the cytotoxic effect of radicicol and cycloproparadicicol analogs on CCRF-CEM cell growth.

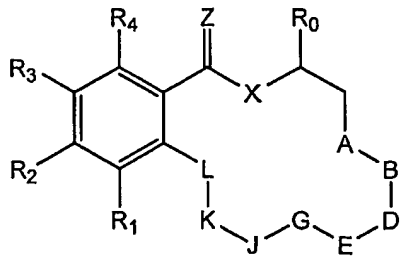
Figure 31 shows Her2 degradation assays.

DESCRIPTION OF THE INVENTION

In recognition of the need to develop novel and effective cancer therapies, the present invention provides novel synthetic methodologies enabling access to macrocycles having a broad range of biological and pharmacological activity. In certain embodiments, the inventive compounds are useful in the treatment of cancer. In certain other embodiments of special interest, the compounds are useful for the treatment of cancers comprising Rb negative cancer cells.

1) General Description of Compounds of the Invention

The compounds of the invention include compounds of the general formula **(II)** as further defined below:



(II)

wherein

R_0 is hydrogen, halogen, cyano, $-OR_Z$, $-N(R_Z)_2$, $-SR_Z$, $-O(C=O)R_Z$, $-N(R_Z)(C=O)(R_Z)$, $-C(O)R_Z$, $-C(O)OR_Z$, $-CON(R_Z)_2$, $-OCO_2R_Z$, or an aliphatic, heteroaliphatic, aryl, heteroaryl, alkylaryl, or alkylheteroaryl moiety, wherein each

occurrence of R_Z is independently hydrogen, a protecting group, or an aliphatic, heteroaliphatic, aryl, heteroaryl, alkylaryl, or alkylheteroaryl moiety

R_1 is hydrogen, halogen, cyano, $-OR_A$, $-N(R_A)_2$, $-SR_A$, $-O(C=O)R_A$, $-N(R_A)(C=O)(R_A)$, $-C(O)R_A$, $-C(O)OR_A$, $-CON(R_A)_2$, $-OCO_2R_A$, or an aliphatic, heteroaliphatic, aryl, heteroaryl, alkylaryl, or alkylheteroaryl moiety, wherein each occurrence of R_A is independently hydrogen, a protecting group, or an aliphatic, heteroaliphatic, aryl, heteroaryl, alkylaryl, or alkylheteroaryl moiety;

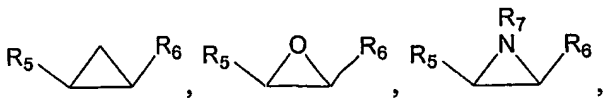
R_2 is hydrogen, halogen, cyano, $-OR_B$, $-N(R_B)_2$, $-SR_B$, $-O(C=O)R_B$, $-N(R_B)(C=O)(R_B)$, $-C(O)R_B$, $-C(O)OR_B$, $-CON(R_B)_2$, $-OCO_2R_B$, or an aliphatic, heteroaliphatic, aryl, heteroaryl, alkylaryl, or alkylheteroaryl moiety, wherein each occurrence of R_B is independently hydrogen, a protecting group or an aliphatic, heteroaliphatic, aryl, heteroaryl, alkylaryl, or alkylheteroaryl moiety;

R_3 is hydrogen, halogen, cyano, $-OR_C$, $-N(R_C)_2$, $-SR_C$, $-O(C=O)R_C$, $-N(R_C)(C=O)(R_C)$, $-C(O)R_C$, $-C(O)OR_C$, $-CON(R_C)_2$, $-OCO_2R_C$, or an aliphatic, heteroaliphatic, aryl, heteroaryl, alkylaryl, or alkylheteroaryl moiety, wherein each occurrence of R_C is independently hydrogen, a protecting group, or an aliphatic, heteroaliphatic, aryl, heteroaryl, alkylaryl, or alkylheteroaryl moiety;

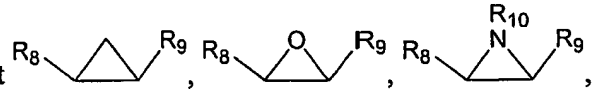
R_4 is hydrogen, halogen, cyano, $-OR_D$, $-N(R_D)_2$, $-SR_D$, $-O(C=O)R_D$, $-N(R_D)(C=O)(R_D)$, $-C(O)R_D$, $-C(O)OR_D$, $-CON(R_D)_2$, $-OCO_2R_D$, or an aliphatic, heteroaliphatic, aryl, heteroaryl, alkylaryl, or alkylheteroaryl moiety, wherein each occurrence of R_D is independently hydrogen, a protecting group or an aliphatic, heteroaliphatic, aryl, heteroaryl, alkylaryl, or alkylheteroaryl moiety;

Z is O, S, or NR_E, wherein R_E is hydrogen, a protecting group, an aliphatic, heteroaliphatic, aryl, heteroaryl, alkylaryl, or alkylheteroaryl moiety, or OR_F, wherein R_F is hydrogen, a protecting group, an aliphatic, heteroaliphatic, aryl, heteroaryl, alkylaryl, or alkylheteroaryl moiety;

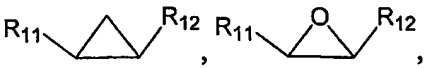
X is O, S or NR_G, wherein R_G is hydrogen or lower alkyl;

A and B together represent 

, -CHR_5-CHR_6-, -CR_5=CR_6-, wherein R_5 and R_6 are each independently hydrogen, halogen, cyano, -OR_J, -$N(R_J)_2$, -SR_J, -O(C=O)R_J, -O(S=O)R_J ,-$N(R_J)$(C=O)(R_J), -C(=O)R_J, -C(=O)OR_J, -$CON(R_J)_2$, -OCO_2R_J, -OS(=O)OR_J or an aliphatic, heteroaliphatic, aryl, heteroaryl, alkylaryl, or alkylheteroaryl moiety, wherein each occurrence of R_J is independently hydrogen, a protecting group, or an aliphatic, heteroaliphatic, aryl, heteroaryl, alkylaryl, or alkylheteroaryl moiety, and wherein R_7 is hydrogen, a protecting group, -OR_K, -SR_K, -C(O)OR_K, -C(O)NR_K, -$S(O)_2R_K$, -O(C=O)R_K, -$N(R_K)$(C=O)(R_K), -C(O)R_K, -C(O)OR_K, -$CON(R_K)_2$, -OCO_2R_K, or an aliphatic, heteroaliphatic, aryl, heteroaryl, alkylaryl, or alkylheteroaryl moiety, wherein each occurrence of R_K is independently hydrogen, a protecting group or an aliphatic, heteroaliphatic, aryl, heteroaryl, alkylaryl, or alkylheteroaryl moiety, or when A and B together represent -CHR_5-CHR_6-, R_5 and R_6 taken together represent a substituted or unsubstituted 3-7 membered aliphatic, heteroaliphatic, aryl or heteroaryl ring;

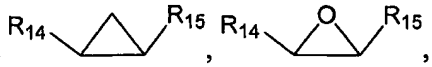
D and E together represent 

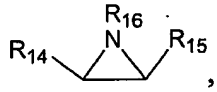
, -CHR_8-CHR_9-, -CR_8=CR_9-, wherein R_8 and R_9 are each independently hydrogen, halogen, cyano, -OR_J, -$N(R_J)_2$, -SR_J, -O(C=O)R_J, -O(S=O)R_J ,-$N(R_J)$(C=O)(R_J), -C(=O)R_J, -C(=O)OR_J, -$CON(R_J)_2$, -OCO_2R_J, -OS(=O)OR_J or an aliphatic, heteroaliphatic, aryl, heteroaryl, alkylaryl, or alkylheteroaryl moiety, wherein each occurrence of R_J is independently hydrogen, a protecting group, or an aliphatic, heteroaliphatic, aryl, heteroaryl, alkylaryl, or alkylheteroaryl moiety, and wherein R_{10} is hydrogen, a protecting group, -OR_K, -SR_K, -C(O)OR_K, -C(O)NR_K, -$S(O)_2R_K$, -O(C=O)R_K, -$N(R_K)$(C=O)(R_K), -C(O)R_K, -C(O)OR_K, -$CON(R_K)_2$, -OCO_2R_K, or an aliphatic, heteroaliphatic, aryl, heteroaryl, alkylaryl, or alkylheteroaryl moiety, wherein each occurrence of R_K is independently hydrogen, a protecting group or an aliphatic, heteroaliphatic, aryl, heteroaryl, alkylaryl, or alkylheteroaryl moiety, or when A and B together represent –CHR_8-CHR_9-, R_9 and R_9 taken together represent a substituted or unsubstituted 3-7 membered aliphatic, heteroaliphatic, aryl or heteroaryl ring;

G and J together represent  ,

$-CHR_{11}-CHR_{12}-$, $-CR_{11}=CR_{12}-$, wherein R_{11} and R_{12} are each independently hydrogen, halogen, cyano, $-OR_J$, $-N(R_J)_2$, $-SR_J$, $-O(C=O)R_J$, $-O(S=O)R_J$,$-N(R_J)(C=O)(R_J)$, $-C(=O)R_J$, $-C(=O)OR_J$,

$-CON(R_J)_2$, $-OCO_2R_J$, $-OS(=O)OR_J$ or an aliphatic, heteroaliphatic, aryl, heteroaryl, alkylaryl, or alkylheteroaryl moiety, wherein each occurrence of R_J is independently hydrogen, a protecting group, or an aliphatic, heteroaliphatic, aryl, heteroaryl, alkylaryl, or alkylheteroaryl moiety, and wherein R_{13} is hydrogen, a protecting group, $-OR_K$, $-SR_K$, $-C(O)OR_K$, $-C(O)NR_K$, $-S(O)_2R_K$, $-O(C=O)R_K$, $-N(R_K)(C=O)(R_K)$, $-C(O)R_K$, $-C(O)OR_K$, $-CON(R_K)_2$, $-OCO_2R_K$, or an aliphatic, heteroaliphatic, aryl, heteroaryl, alkylaryl, or alkylheteroaryl moiety, wherein each occurrence of R_K is independently hydrogen, a protecting group or an aliphatic, heteroaliphatic, aryl, heteroaryl, alkylaryl, or alkylheteroaryl moiety, or when A and B together represent $-CHR_{11}-CHR_{12}-$, R_{11} and R_{12} taken together represent a substituted or unsubstituted 3-7 membered aliphatic, heteroaliphatic, aryl or heteroaryl ring;

K and L together represent  ,



$-CHR_{14}-CHR_{15}-$, $-CR_{14}=CR_{15}-$, wherein R_{14} and R_{15} are each independently hydrogen, halogen, cyano, $-OR_J$, $-N(R_J)_2$, $-SR_J$, $-O(C=O)R_J$, $-O(S=O)R_J$,$-N(R_J)(C=O)(R_J)$, $-C(=O)R_J$, $-C(=O)OR_J$,

$-CON(R_J)_2$, $-OCO_2R_J$, $-OS(=O)OR_J$ or an aliphatic, heteroaliphatic, aryl, heteroaryl, alkylaryl, or alkylheteroaryl moiety, wherein each occurrence of R_J is independently hydrogen, a protecting group, or an aliphatic, heteroaliphatic, aryl, heteroaryl, alkylaryl, or alkylheteroaryl moiety, and wherein R_{16} is hydrogen, a protecting group, $-OR_K$, $-SR_K$, $-C(O)OR_K$, $-C(O)NR_K$, $-S(O)_2R_K$, $-O(C=O)R_K$, $-N(R_K)(C=O)(R_K)$, $-C(O)R_K$, $-C(O)OR_K$, $-CON(R_K)_2$, $-OCO_2R_K$, or an aliphatic, heteroaliphatic, aryl, heteroaryl, alkylaryl, or alkylheteroaryl moiety, wherein each occurrence of R_K is independently hydrogen, a protecting group or an aliphatic, heteroaliphatic, aryl, heteroaryl, alkylaryl,

or alkylheteroaryl moiety, or when A and B together represent $-CHR_{14}-CHR_{15}-$, R_{14} and R_{15} taken together represent a substituted or unsubstituted 3-7 membered aliphatic, heteroaliphatic, aryl or heteroaryl ring;

whereby each of the foregoing aliphatic and heteroaliphatic moieties may independently be substituted or unsubstituted, cyclic or acyclic, or branched or unbranched, and each aryl, heteroaryl, alkylaryl, and alkylheteroaryl moiety may be substituted or unsubstituted; and

pharmaceutically acceptable derivatives thereof.

In certain other embodiments of the invention the compounds are subject to one or more, or all of the following limitations:

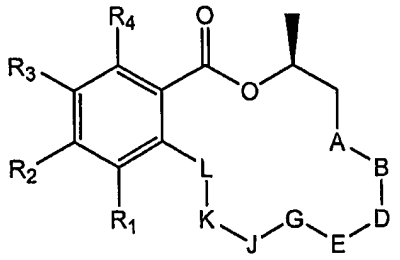
if Z is O; if X is O; if R_0 is methyl; if A and B together are $-CR_4=CR_5-$ and R_5 and R_6 are each hydrogen; if D and E together are –COH=COH-; if G and J together are $-CH_2-CH_2-$; if K and L together are -CH=CH-; if R_1 is hydrogen; and if R_3 is hydrogen;

then R_2 and R_4 are each not $-OR_B$, wherein R_B is hydrogen or an alkyl, alkoxy, alkenyl, alkenyloxy, alkynyl, aryl, aryloxy, heterocycle, cycloalkyl, cycloalkenyl, or cycloalkenyl fused to an aryl group.

In certain embodiments of the invention, the genuses or subclasses of compounds of the invention exclude aigailomycins A-E.

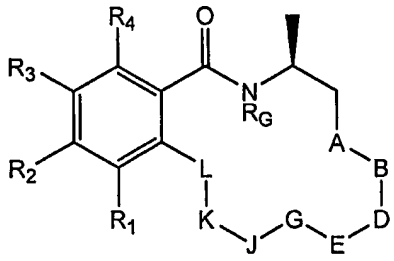
2) Featured Classes of Compounds

It will be appreciated that for compounds as generally described above, certain classes of compounds are of special interest. For example, one class of compounds of special interest includes those compounds having the structure of formula (II) in which Z and X are each O, and the compound has the structure:



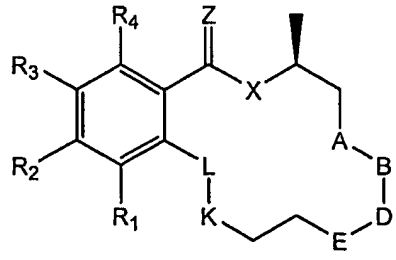
and R_1, R_2, R_3, R_4, A-B, D-E, G-J, and K-L are as defined above and in subclasses herein.

Another class of compounds of special interest consists of compounds having the structure of formula (I) in which Z is O and X is NR_G, and the compound has the structure:



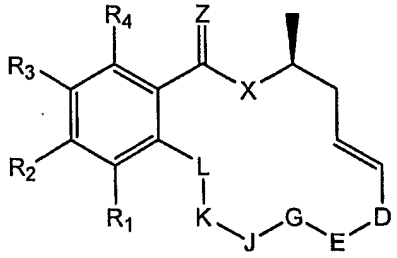
and R_1, R_2, R_3, R_4, R_G, A-B, D-E, G-J, and K-L are as defined above and in subclasses herein.

Another class of compounds of special interest consists of compounds having the structure of formula (I) in which G and J together represent $-CH_2-CH_2-$ and the compound has the structure:



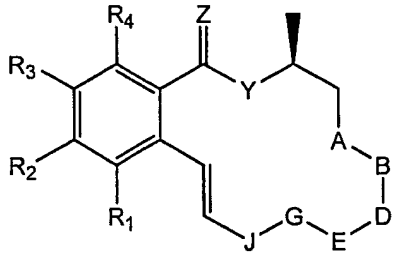
and R_1, R_2, R_3, R_4, Z, X, A-B, D-E, and K-L are as defined above and in subclasses herein.

Another class of compounds of special interest consists of compounds having the structure of formula (I) in which A and B together represent -CH=CH- and the compound has the structure:



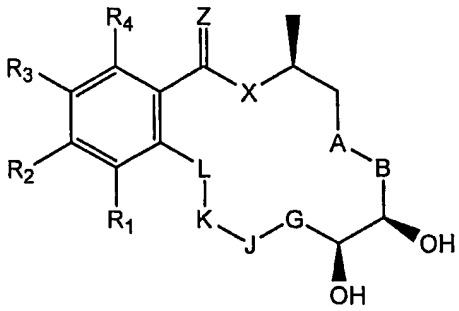
and R_1, R_2, R_3, R_4, R_5, R_6, Z, X, D-E, G-J, and K-L are as defined above and in subclasses herein.

Another class of compounds of special interest consists of compounds having the structure of formula (I) in which K and L together represent -CH=CH- and the compound has the structure:



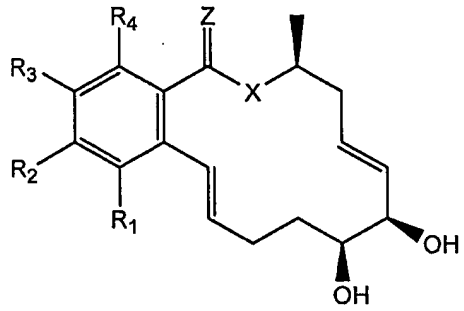
and R_1, R_2, R_3, R_4, Z, X, A-B, D-E, and G-J are as defined above and in subclasses herein.

Another class of compounds of special interest consists of compounds having the structure of formula (I) in which D and E together represent –CHOH=CHOH- and the compound has the structure:



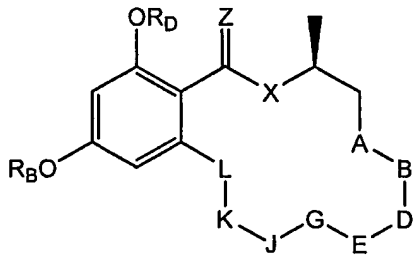
and R_1, R_2, R_3, R_4, R_7, Z, X, A-B, G-J, and K-L are as defined above and in subclasses herein.

Another class of compounds of special interest consists of compounds having the structure of formula (I) in which the compound has the structure:



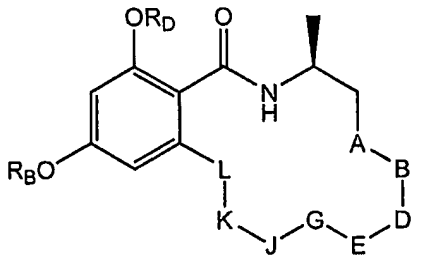
and R_1, R_2, R_3, R_4, Z, and X are as defined above and in subclasses herein. In certain embodiments, Z is oxygen and X is NR_G, wherein R_G is defined above. In other embodiments, Z is oxygen an X is NH.

Another class of compounds of special interest consists of compounds having the structure of formula (I) in which the compound has the structure:



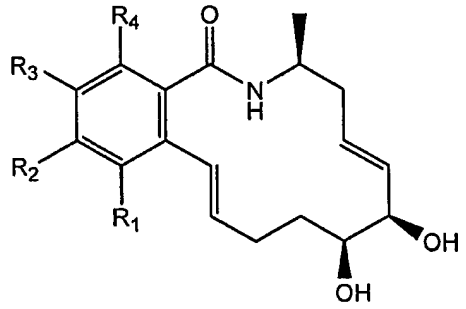
and Z, X, A-B, D-E, G-J, and K-L are as defined above and in subclasses herein. In certain embodiments, R_D and R_B are each hydrogen.

Another class of compounds of special interest consists of compounds having the structure of formula (I) in which the compound has the structure:



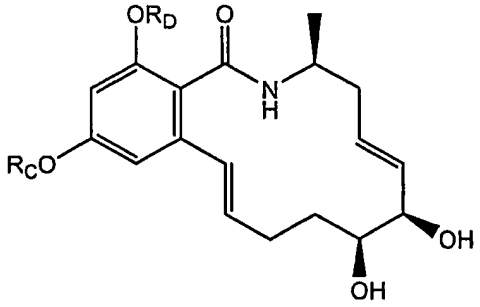
and R_D, R_B, A-B, D-E, G-J, and K-L are as defined above and in subclasses herein.

Another class of compounds of special interests consists of compounds having the structure of formula (I) in which the compound has the structure:



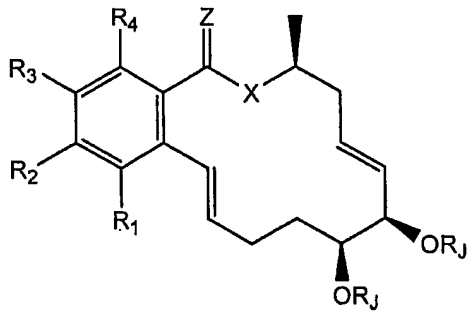
and R_1, R_2, R_3, and R_4 are as defined above and in subclasses herein.

Another class of compounds of special interest consists of compounds having the structure of formula (I) in which the compound has the structure:



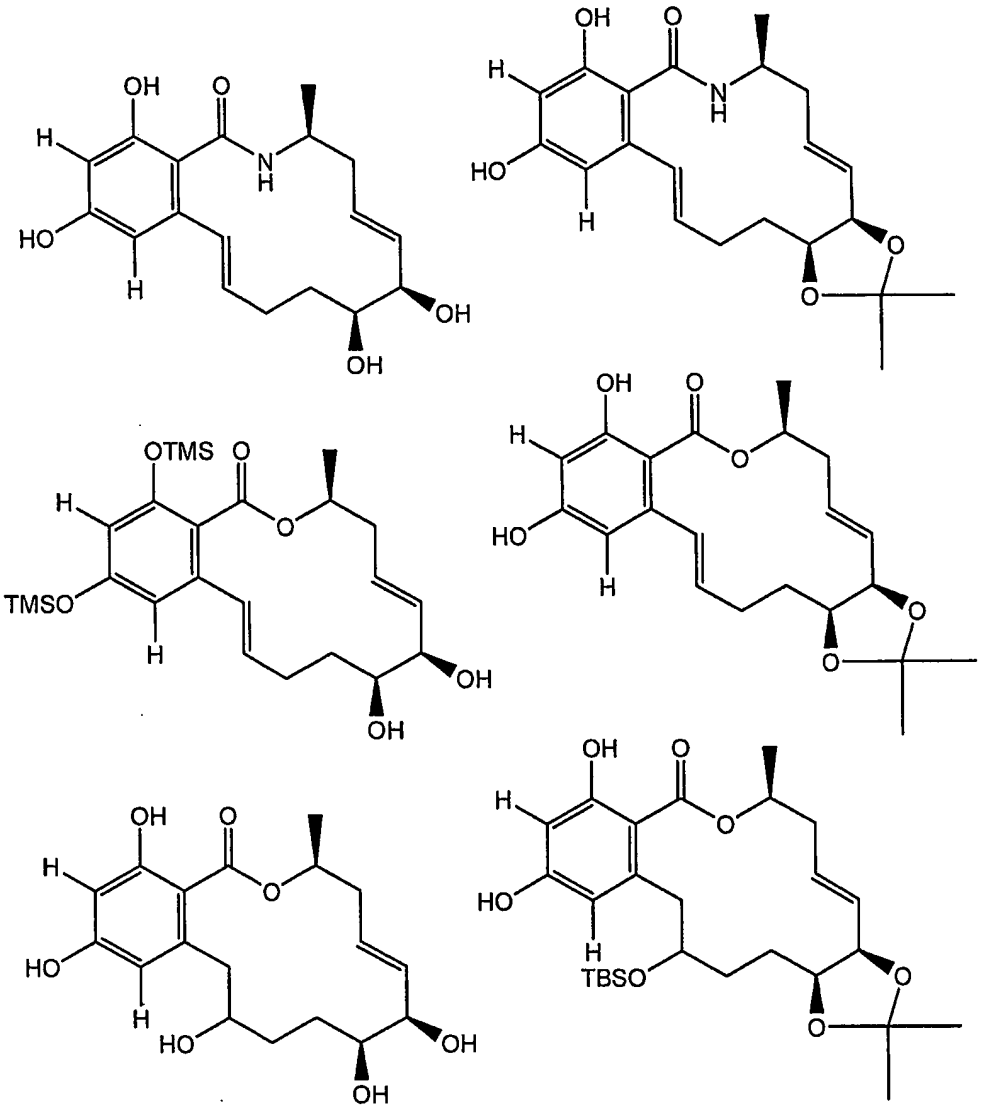
and R_C and R_D are as defined above and in subclasses herein. In certain embodiments, R_D and R_C are each hydrogen.

Another class of compounds of special interest consists of compounds having the structure of formula (I) in which the compound has the structure:



and R_1, R_2, R_3, R_4, R_J, Z, and X are as defined above and in subclasses herein. In certain embodiments, Z is oxygen and X is oxygen. In other embodiments, Z is oxygen and X is NH.

The following structures illustrate several exemplary types of compounds of these classes. Others will be readily apparent to the reader.



A number of important subclasses of each of the foregoing classes deserve separate mention; these subclasses include subclasses of the foregoing classes in which:

i) Z and X are each O;

ii) Z is O and X is NH;

iii) A and B together are a trans carbon-carbon double bond;

iv) A and B together are a carbon-carbon single bond;

v) D and E together are –CHOH-CHOH-;

vi) J and G together are a carbon-carbon single bond;

vii) K and L together are a trans carbon-carbon double bond;

viii) R_2 and R_4 are each independently hydrogen, halogen, cyano, $-OR_J$, $-N(R_J)_2$, $-SR_J$,

$-O(C=O)R_J$, $-O(S=O)R_J$, $-N(R_J)(C=O)(R_J)$, or $-OCO_2R_J$, $-OSO_2R_J$, and each occurrence of R_J is independently hydrogen, a protecting group or an aliphatic, heteroaliphatic, aryl, heteroaryl, alkylaryl, or alkylheteroaryl moiety;

ix) R_2 and R_4 are each independently hydroxy;

x) D and E together are $-CHR_8-CHR_9-$;

xi) D and E together are $-CR_8=CR_9-$;

xii) R_1 and R_3 are each hydrogen;

xiii) G and J together are $-CHR_{10}-CHR_{11}-$;

xiv) G and J together are $-CR_{10}=CR_{11}-$;

xv) R_{10} and R_{11} are each hydrogen;

xvi) Z is O;

xvii) Z is S;

xviii) X is S;

xix) X is NR_G;

xx) X is O;

xxi) R_1 is hydrogen, halogen, lower alkyl, lower heteroalkyl, lower alkylaryl, lower alkylheteroaryl, or $N(R_A)_2$, wherein each occurrence of R_A is independently hydrogen, a protecting group, or an aliphatic, heteroaliphatic, aryl, heteroaryl, alkylaryl, or alkylheteroaryl moiety and R_3 is hydrogen, halogen, lower alkyl, lower heteroalkyl, lower alkylaryl, lower alkylheteroaryl, or $-N(R_C)_2$, wherein each occurrence of R_C is independently hydrogen, a protecting group, or an aliphatic, heteroaliphatic, aryl, heteroaryl, alkylaryl, or alkylheteroaryl moiety;

xxii) R_1 and R_3 are each independently halogen, hydrogen, or lower alkyl;

xxiii) R_2 is hydrogen, halogen, $-OR_B$, $-N(R_B)_2$, $-SR_B$, $-O(C=O)R_B$, $-N(R_B)(C=O)(R_B)$,

$-C(O)R_B$, $-C(O)OR_B$, $-CON(R_B)_2$, $-OCO_2R_B$, or lower alkyl, lower heteroalkyl, lower alkylaryl, lower alkylheteroaryl, wherein each occurrence of R_B is independently hydrogen, a protecting group or an aliphatic, heteroaliphatic, aryl, heteroaryl, alkylaryl,

or alkylheteroaryl moiety, and R_4 is hydrogen, halogen, $-OR_D$, $-N(R_D)_2$, $-SR_D$, $-O(C=O)R_D$, $-N(R_D)(C=O)(R_D)$, $-C(O)R_D$, $-C(O)OR_D$, $-CON(R_D)_2$, $-OCO_2R_D$, or lower alkyl, lower heteroalkyl, lower alkylaryl, lower alkylheteroaryl, wherein each occurrence of R_D is independently hydrogen, a protecting group or an aliphatic, heteroaliphatic, aryl, heteroaryl, alkylaryl, or alkylheteroaryl moiety;

xxiv) R_2 is hydrogen or $-OR_B$, wherein each occurrence of R_B is independently hydrogen, a protecting group or an aliphatic, heteroaliphatic, aryl, heteroaryl, alkylaryl, or alkylheteroaryl moiety, and R_4 is hydrogen or $-OR_D$, wherein each occurrence of R_D is independently hydrogen, a protecting group or an aliphatic, heteroaliphatic, aryl, heteroaryl, alkylaryl, or alkylheteroaryl moiety;

xxv) R_0 is hydrogen or aliphatic;

xxvi) R_0 is methyl;

xxvii) K and L together are a cis carbon-carbon double bond.

In certain embodiments of the compounds described above, R_1 and R_3 are each independently halogen, hydrogen, or lower alkyl; R_2 is hydrogen or $-OR_B$, wherein each occurrence of R_B is independently hydrogen, a protecting group or an aliphatic, heteroaliphatic, aryl, heteroaryl, alkylaryl, or alkylheteroaryl moiety, and R_4 is hydrogen or $-OR_D$, wherein each occurrence of R_D is independently hydrogen, a protecting group or an aliphatic, heteroaliphatic, aryl, heteroaryl, alkylaryl, or alkylheteroaryl moiety.

Some of the foregoing compounds can exist in various isomeric forms. The invention encompasses the compounds as individual isomers substantially free of other isomers and alternatively, as mixtures of various isomers, e.g., racemic mixtures of stereoisomers, cis and trans isomers. The invention also encompasses tautomers of specific compounds as described above. In addition to the above-mentioned compounds per se, this invention also encompasses pharmaceutically acceptable derivatives of these compounds and compositions comprising one or more compounds of the invention and one or more pharmaceutically acceptable excipients or additives.

Compounds of this invention which are of particular interest include those which:

- exhibit cytotoxic or growth inhibitory effect on cancer cell lines maintained in vitro or in animal studies using a scientifically acceptable cancer cell xenograft model;
- exhibit cytotoxic or growth inhibitory effect on cancer cell lines comprising Rb negative cells;
- exhibit antimalarial activity;
- exhibit antibacterial activity;
- bind to and/or inhibit the Hsp90 family of chaperones;
- exhibit cytotoxic or growth inhibitory effect on cancer cell lines comprising Rb positive cells.

This invention also provides a pharmaceutical preparation comprising at least one of the compounds as described above and herein, or a pharmaceutically acceptable derivative thereof, which compounds are capable of inhibiting the growth of or killing cancer cells, and, in certain embodiments of special interest are capable of inhibiting the growth of or killing cancer cells.

The invention further provides a method for inhibiting tumor growth and/or tumor metastasis. The method involves the administration of a therapeutically effective amount of the compound or a pharmaceutically acceptable derivative thereof to a subject (including, but not limited to a human or animal) in need of it. In certain embodiments, the inventive compounds are useful for the treatment of solid tumors. In still other embodiments of interest, the inventive compounds are useful for the treatment of glioblastoma, retinoblastoma or small cell lung cancer.

3) Compounds and Definitions

As discussed above, this invention provides novel compounds with a range of biological properties. Compounds of this invention have biological activities relevant for the treatment of diseases or other disorders such as proliferative diseases, including,

but not limited to cancer. More generally, the compounds are useful in the regulation of the cell cycle pathway.

Compounds of this invention include those specifically set forth above and described herein, and are illustrated in part by the various classes, subgenera and species disclosed elsewhere herein.

It will be appreciated by one of ordinary skill in the art that asymmetric centers may exist in the compounds of the present invention. Thus, inventive compounds and pharmaceutical compositions thereof may be in the form of an individual enantiomer, diastereomer or geometric isomer, or may be in the form of a mixture of stereoisomers. In certain embodiments, the compounds of the invention are enantiopure compounds. In certain other embodiments, a mixtures of stereoisomers or diastereomers are provided.

Additionally, the present invention provides pharmaceutically acceptable derivatives of the inventive compounds, and methods of treating a subject using these compounds, pharmaceutical compositions thereof, or either of these in combination with one or more additional therapeutic agents. The phrase, "pharmaceutically acceptable derivative", as used herein, denotes any pharmaceutically acceptable salt, ester, or salt of such ester, of such compound, or any other adduct or derivative which, upon administration to a patient, is capable of providing (directly or indirectly) a compound as otherwise described herein, or a metabolite or residue thereof. Pharmaceutically acceptable derivatives thus include among others pro-drugs. A pro-drug is a derivative of a compound, usually with significantly reduced pharmacological activity, which contains an additional moiety which is susceptible to removal *in vivo* yielding the parent molecule as the pharmacologically active species. An example of a pro-drug is an ester which is cleaved in vivo to yield a compound of interest. Pro-drugs of a variety of compounds, and materials and methods for derivatizing the parent compounds to create the pro-drugs, are known and may be adapted to the present invention. Certain exemplary pharmaceutical compositions and pharmaceutically acceptable derivatives will be discussed in more detail herein below.

Certain compounds of the present invention, and definitions of specific functional groups are also described in more detail below. For purposes of this invention, the chemical elements are identified in accordance with the Periodic Table of

the Elements, CAS version, Handbook of Chemistry and Physics, 75th Ed., inside cover, and specific functional groups are generally defined as described therein. Additionally, general principles of organic chemistry, as well as specific functional moieties and reactivity, are described in "Organic Chemistry", Thomas Sorrell, University Science Books, Sausalito: 1999, the entire contents of which are incorporated herein by reference. Furthermore, it will be appreciated by one of ordinary skill in the art that the synthetic methods, as described herein, utilize a variety of protecting groups. By the term "protecting group", has used herein, it is meant that a particular functional moiety, e.g., O, S, or N, is temporarily blocked so that a reaction can be carried out selectively at another reactive site in a multifunctional compound. In preferred embodiments, a protecting group reacts selectively in good yield to give a protected substrate that is stable to the projected reactions; the protecting group must be selectively removed in good yield by readily available, preferably nontoxic reagents that do not attack the other funcational groups; the protecting group forms an easily separable derivative (more preferably without the generation of new stereogenic centers); and the protecting group has a minimum of additional functionality to avoid further sites of reaction. As detailed herein, oxygen, sulfur, nitrogen and carbon protecting groups may be utilized. Exemplary protecting groups are detailed herein, however, it will be appreciated that the present invention is not intended to be limited to these protecting groups; rather, a variety of additional equivalent protecting groups can be readily identified using the above criteria and utilized in the method of the present invention. Additionally, a variety of protecting groups are described in "Protective Groups in Organic Synthesis" Third Ed. Greene, T.W. and Wuts, P.G., Eds., John Wiley & Sons, New York: 1999, the entire contents of which are hereby incorporated by reference.

It will be appreciated that the compounds, as described herein, may be substituted with any number of substituents or functional moieties. In general, the term "substituted" whether preceded by the term "optionally" or not, and substituents contained in formulas of this invention, refer to the replacement of hydrogen radicals in a given structure with the radical of a specified substituent. When more than one position in any given structure may be substituted with more than one substituent selected from a specified group, the substituent may be either the same or different at

every position. As used herein, the term "substituted" is contemplated to include all permissible substituents of organic compounds. In a broad aspect, the permissible substituents include acyclic and cyclic, branched and unbranched, carbocyclic and heterocyclic, aromatic and nonaromatic substituents of organic compounds. For purposes of this invention, heteroatoms such as nitrogen may have hydrogen substituents and/or any permissible substituents of organic compounds described herein which satisfy the valencies of the heteroatoms. Furthermore, this invention is not intended to be limited in any manner by the permissible substituents of organic compounds. Combinations of substituents and variables envisioned by this invention are preferably those that result in the formation of stable compounds useful in the treatment, for example of proliferative disorders, including, but not limited to cancer. The term "stable", as used herein, preferably refers to compounds which possess stability sufficient to allow manufacture and which maintain the integrity of the compound for a sufficient period of time to be detected and preferably for a sufficient period of time to be useful for the purposes detailed herein.

The term "aliphatic", as used herein, includes both saturated and unsaturated, straight chain (*i.e.*, unbranched), branched, cyclic, or polycyclic aliphatic hydrocarbons, which are optionally substituted with one or more functional groups. As will be appreciated by one of ordinary skill in the art, "aliphatic" is intended herein to include, but is not limited to, alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, and cycloalkynyl moieties. Thus, as used herein, the term "alkyl" includes straight, branched and cyclic alkyl groups. An analogous convention applies to other generic terms such as "alkenyl", "alkynyl" and the like. Furthermore, as used herein, the terms "alkyl", "alkenyl", "alkynyl" and the like encompass both substituted and unsubstituted groups. In certain embodiments, as used herein, "lower alkyl" is used to indicate those alkyl groups (cyclic, acyclic, substituted, unsubstituted, branched or unbranched) having 1-6 carbon atoms.

In certain embodiments, the alkyl, alkenyl and alkynyl groups employed in the invention contain 1-20 aliphatic carbon atoms. In certain other embodiments, the alkyl, alkenyl, and alkynyl groups employed in the invention contain 1-10 aliphatic carbon atoms. In yet other embodiments, the alkyl, alkenyl, and alkynyl groups employed in the invention contain 1-8 aliphatic carbon atoms. In still other embodiments, the alkyl,

alkenyl, and alkynyl groups employed in the invention contain 1-6 aliphatic carbon atoms. In yet other embodiments, the alkyl, alkenyl, and alkynyl groups employed in the invention contain 1-4 carbon atoms. Illustrative aliphatic groups thus include, but are not limited to, for example, methyl, ethyl, n-propyl, isopropyl, cyclopropyl, -CH_2-cyclopropyl, allyl, n-butyl, sec-butyl, isobutyl, tert-butyl, cyclobutyl, -CH_2-cyclobutyl, n-pentyl, sec-pentyl, isopentyl, tert-pentyl, cyclopentyl, -CH_2-cyclopentyl, n-hexyl, sec-hexyl, cyclohexyl, -CH_2-cyclohexyl moieties and the like, which again, may bear one or more substituents. Alkenyl groups include, but are not limited to, for example, ethenyl, propenyl, butenyl, 1-methyl-2-buten-1-yl, and the like. Representative alkynyl groups include, but are not limited to, ethynyl, 2-propynyl (propargyl), 1-propynyl and the like.

The term "alkoxy", or "thioalkyl" as used herein refers to an alkyl group, as previously defined, attached to the parent molecular moiety through an oxygen atom or through a sulfur atom. In certain embodiments, the alkyl group contains 1-20 alipahtic carbon atoms. In certain other embodiments, the alkyl group contains 1-10 aliphatic carbon atoms. In yet other embodiments, the alkyl, alkenyl, and alkynyl groups employed in the invention contain 1-8 aliphatic carbon atoms. In still other embodiments, the alkyl group contains 1-6 aliphatic carbon atoms. In yet other embodiments, the alkyl group contains 1-4 aliphatic carbon atoms. Examples of alkoxy, include but are not limited to, methoxy, ethoxy, propoxy, isopropoxy, n-butoxy, tert-butoxy, neopentoxy and n-hexoxy. Examples of thioalkyl include, but are not limited to, methylthio, ethylthio, propylthio, isopropylthio, n-butylthio, and the like.

The term "alkylamino" refers to a group having the structure -NHR' wherein R' is alkyl, as defined herein. In certain embodiments, the alkyl group contains 1-20 aliphatic carbon atoms. In certain other embodiments, the alkyl group contains 1-10 aliphatic carbon atoms. In yet other embodiments, the alkyl, alkenyl, and alkynyl groups employed in the invention contain 1-8 aliphatic carbon atoms. In still other embodiments, the alkyl group contains 1-6 aliphatic carbon atoms. In yet other embodiments, the alkyl group contains 1-4 aliphatic carbon atoms. Examples of alkylamino include, but are not limited to, methylamino, ethylamino, iso-propylamino and the like.

Some examples of substituents of the above-described aliphatic (and other) moieties of compounds of the invention include, but are not limited to aliphatic; heteroaliphatic; aryl; heteroaryl; alkylaryl; alkylheteroaryl; alkoxy; aryloxy; heteroalkoxy; heteroaryloxy; alkylthio; arylthio; heteroalkylthio; heteroarylthio; F; Cl; Br; I; -OH; $-NO_2$; -CN; $-CF_3$; $-CH_2CF_3$; $-CHCl_2$; $-CH_2OH$; $-CH_2CH_2OH$; $-CH_2NH_2$; $-CH_2SO_2CH_3$; $-C(O)R_x$; $-CO_2(R_x)$; $-CON(R_x)_2$; $-OC(O)R_x$; $-OCO_2R_x$; $-OCON(R_x)_2$; $-N(R_x)_2$; $-S(O)_2R_x$; $-NR_x(CO)R_x$ wherein each occurrence of R_x independently includes, but is not limited to, aliphatic, heteroaliphatic, aryl, heteroaryl, alkylaryl, or alkylheteroaryl, wherein any of the aliphatic, heteroaliphatic, alkylaryl, or alkylheteroaryl substituents described above and herein may be substituted or unsubstituted, branched or unbranched, cyclic or acyclic, and wherein any of the aryl or heteroaryl substituents described above and herein may be substituted or unsubstituted. Additional examples of generally applicable substituents are illustrated by the specific embodiments shown in the Examples that are described herein.

In general, the terms "aryl" and "heteroaryl", as used herein, refer to stable mono- or polycyclic, heterocyclic, polycyclic, and polyheterocyclic unsaturated moieties having preferably 3-14 carbon atoms, each of which may be substituted or unsubstituted. Substituents include, but are not limited to, any of the previously mentioned substitutents, i.e., the substituents recited for aliphatic moieties, or for other moieties as disclosed herein, resulting in the formation of a stable compound. In certain embodiments of the present invention, "aryl" refers to a mono- or bicyclic carbocyclic ring system having one or two aromatic rings including, but not limited to, phenyl, naphthyl, tetrahydronaphthyl, indanyl, indenyl and the like. In certain embodiments of the present invention, the term "heteroaryl", as used herein, refers to a cyclic aromatic radical having from five to ten ring atoms of which one ring atom is selected from S, O and N; zero, one or two ring atoms are additional heteroatoms independently selected from S, O and N; and the remaining ring atoms are carbon, the radical being joined to the rest of the molecule via any of the ring atoms, such as, for example, pyridyl, pyrazinyl, pyrimidinyl, pyrrolyl, pyrazolyl, imidazolyl, thiazolyl, oxazolyl, isooxazolyl, thiadiazolyl, oxadiazolyl, thiophenyl, furanyl, quinolinyl, isoquinolinyl, and the like.

It will be appreciated that aryl and heteroaryl groups (including bicyclic aryl groups) can be unsubstituted or substituted, wherein substitution includes replacement of one, two or three of the hydrogen atoms thereon independently with any one or more of the following moieties including, but not limited to: aliphatic; heteroaliphatic; aryl; heteroaryl; alkylaryl; alkylheteroaryl; alkoxy; aryloxy; heteroalkoxy; heteroaryloxy; alkylthio; arylthio; heteroalkylthio; heteroarylthio; F; Cl; Br; I; -OH; $-NO_2$; -CN; $-CF_3$; $-CH_2CF_3$; $-CHCl_2$; $-CH_2OH$; $-CH_2CH_2OH$; $-CH_2NH_2$; $-CH_2SO_2CH_3$; $-C(O)R_x$; $-CO_2(R_x)$; $-CON(R_x)_2$; $-OC(O)R_x$; $-OCO_2R_x$; $-OCON(R_x)_2$; $-N(R_x)_2$; $-S(O)_2R_x$; $-NR_x(CO)R_x$ wherein each occurrence of R_x independently includes, but is not limited to, aliphatic, heteroaliphatic, aryl, heteroaryl, alkylaryl, or alkylheteroaryl, wherein any of the aliphatic, heteroaliphatic, alkylaryl, or alkylheteroaryl substituents described above and herein may be substituted or unsubstituted, branched or unbranched, cyclic or acyclic, and wherein any of the aryl or heteroaryl substituents described above and herein may be substituted or unsubstituted. Additional examples of generally applicable substitutents are illustrated by the specific embodiments shown in the Examples that are described herein.

The term "cycloalkyl", as used herein, refers specifically to groups having three to seven, preferably three to ten carbon atoms. Suitable cycloalkyls include, but are not limited to cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl and the like, which, as in the case of other aliphatic, heteroaliphatic or hetercyclic moieties, may optionally be substituted with substituents including, but not limited to aliphatic; heteroaliphatic; aryl; heteroaryl; alkylaryl; alkylheteroaryl; alkoxy; aryloxy; heteroalkoxy; heteroaryloxy; alkylthio; arylthio; heteroalkylthio; heteroarylthio; F; Cl; Br; I; -OH; $-NO_2$; -CN; $-CF_3$; $-CH_2CF_3$; $-CHCl_2$; $-CH_2OH$; $-CH_2CH_2OH$; $-CH_2NH_2$; $-CH_2SO_2CH_3$; $-C(O)R_x$; $-CO_2(R_x)$; $-CON(R_x)_2$; $-OC(O)R_x$; $-OCO_2R_x$; $-OCON(R_x)_2$; $-N(R_x)_2$; $-S(O)_2R_x$; $-NR_x(CO)R_x$ wherein each occurrence of R_x independently includes, but is not limited to, aliphatic, heteroaliphatic, aryl, heteroaryl, alkylaryl, or alkylheteroaryl, wherein any of the aliphatic, heteroaliphatic, alkylaryl, or alkylheteroaryl substituents described above and herein may be substituted or unsubstituted, branched or unbranched, cyclic or acyclic, and wherein any of the aryl or heteroaryl substituents described above and herein may be substituted or unsubstituted.

Additional examples of generally applicable substitutents are illustrated by the specific embodiments shown in the Examples that are described herein.

The term "heteroaliphatic", as used herein, refers to aliphatic moieties which contain one or more oxygen, sulfur, nitrogen, phosphorus or silicon atoms, *e.g.*, in place of carbon atoms. Heteroaliphatic moieties may be branched, unbranched, cyclic or acyclic and include saturated and unsaturated heterocycles such as morpholino, pyrrolidinyl, etc. In certain embodiments, heteroaliphatic moieties are substituted by independent replacement of one or more of the hydrogen atoms thereon with one or more moieties including, but not limited to aliphatic; heteroaliphatic; aryl; heteroaryl; alkylaryl; alkylheteroaryl; alkoxy; aryloxy; heteroalkoxy; heteroaryloxy; alkylthio; arylthio; heteroalkylthio; heteroarylthio; F; Cl; Br; I; -OH; $-NO_2$; -CN; $-CF_3$; $-CH_2CF_3$; $-CHCl_2$; $-CH_2OH$; $-CH_2CH_2OH$; $-CH_2NH_2$; $-CH_2SO_2CH_3$; $-C(O)R_x$; $-CO_2(R_x)$; $-CON(R_x)_2$; $-OC(O)R_x$; $-OCO_2R_x$; $-OCON(R_x)_2$; $-N(R_x)_2$; $-S(O)_2R_x$; $-NR_x(CO)R_x$ wherein each occurrence of R_x independently includes, but is not limited to, aliphatic, heteroaliphatic, aryl, heteroaryl, alkylaryl, or alkylheteroaryl, wherein any of the aliphatic, heteroaliphatic, alkylaryl, or alkylheteroaryl substituents described above and herein may be substituted or unsubstituted, branched or unbranched, cyclic or acyclic, and wherein any of the aryl or heteroaryl substituents described above and herein may be substituted or unsubstituted. Additional examples of generally applicable substitutents are illustrated by the specific embodiments shown in the Examples that are described herein.

The terms "halo" and "halogen" as used herein refer to an atom selected from fluorine, chlorine, bromine and iodine.

The term "haloalkyl" denotes an alkyl group, as defined above, having one, two, or three halogen atoms attached thereto and is exemplified by such groups as chloromethyl, bromoethyl, trifluoromethyl, and the like.

The term "heterocycloalkyl" or "heterocycle", as used herein, refers to a non-aromatic 5-, 6- or 7- membered ring or a polycyclic group, including, but not limited to a bi- or tri-cyclic group comprising fused six-membered rings having between one and three heteroatoms independently selected from oxygen, sulfur and nitrogen, wherein (i) each 5-membered ring has 0 to 1 double bonds and each 6-membered ring has 0 to 2 double bonds, (ii) the nitrogen and sulfur heteroatoms may be optionally be oxidized,

(iii) the nitrogen heteroatom may optionally be quaternized, and (iv) any of the above heterocyclic rings may be fused to a benzene ring. Representative heterocycles include, but are not limited to, pyrrolidinyl, pyrazolinyl, pyrazolidinyl, imidazolinyl, imidazolidinyl, piperidinyl, piperazinyl, oxazolidinyl, isoxazolidinyl, morpholinyl, thiazolidinyl, isothiazolidinyl, and tetrahydrofuryl. In certain embodiments, a "substituted heterocycloalkyl or heterocycle" group is utilized and as used herein, refers to a heterocycloalkyl or heterocycle group, as defined above, substituted by the independent replacement of one, two or three of the hydrogen atoms thereon with but are not limited to aliphatic; heteroaliphatic; aryl; heteroaryl; alkylaryl; alkylheteroaryl; alkoxy; aryloxy; heteroalkoxy; heteroaryloxy; alkylthio; arylthio; heteroalkylthio; heteroarylthio; F; Cl; Br; I; -OH; $-NO_2$; -CN; $-CF_3$; $-CH_2CF_3$; $-CHCl_2$; $-CH_2OH$; $-CH_2CH_2OH$; $-CH_2NH_2$; $-CH_2SO_2CH_3$; $-C(O)R_x$; $-CO_2(R_x)$; $-CON(R_x)_2$; $-OC(O)R_x$; $-OCO_2R_x$; $-OCON(R_x)_2$; $-N(R_x)_2$; $-S(O)_2R_x$; $-NR_x(CO)R_x$ wherein each occurrence of R_x independently includes, but is not limited to, aliphatic, heteroaliphatic, aryl, heteroaryl, alkylaryl, or alkylheteroaryl, wherein any of the aliphatic, heteroaliphatic, alkylaryl, or alkylheteroaryl substituents described above and herein may be substituted or unsubstituted, branched or unbranched, cyclic or acyclic, and wherein any of the aryl or heteroaryl substituents described above and herein may be substituted or unsubstituted. Additional examples of generally applicable substitutents are illustrated by the specific embodiments shown in the Examples which are described herein.

4) Synthetic Methodology

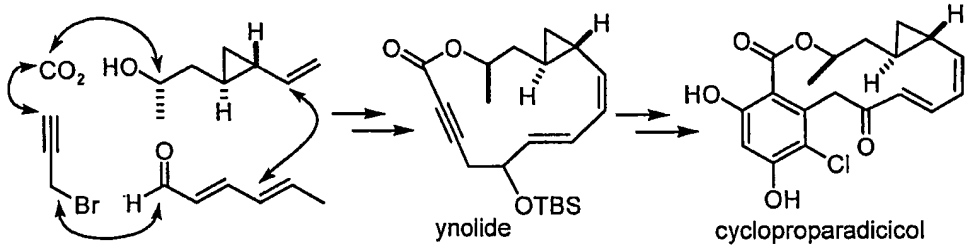
Access to resorcyclic macrolides such as radicicol and monocillin was previously limited to compounds accessed via the natural products (see, for example, US Patent 5,650,430; US Patent 5,731,343; US Patent 6,239,168; US Patent 5,977,165; and US Patent 5,597,846; each of which is incorporated herein by reference). An efficient and practical synthesis of radicicol and monocillin was previously described in USSN 09/938,754, filed August 25, 2001, which is incorporated herein by reference. In recognition of the need for a more efficient and practical route to this class of therapeutic agents, the present invention also provides novel synthetic methodologies for the synthesis of radicicol, monocillin, and analogues thereof as well as aigialomycins and analogues thereof. Although the synthesis of radicicol and

aigialomycin D is described specifically herein directly below (and in the Examples), it will be appreciated that this methodology is generally applicable to the generation of analogues and conjugates as discussed in more detail after the discussion of the synthesis of radicicol and aigialomycin D.

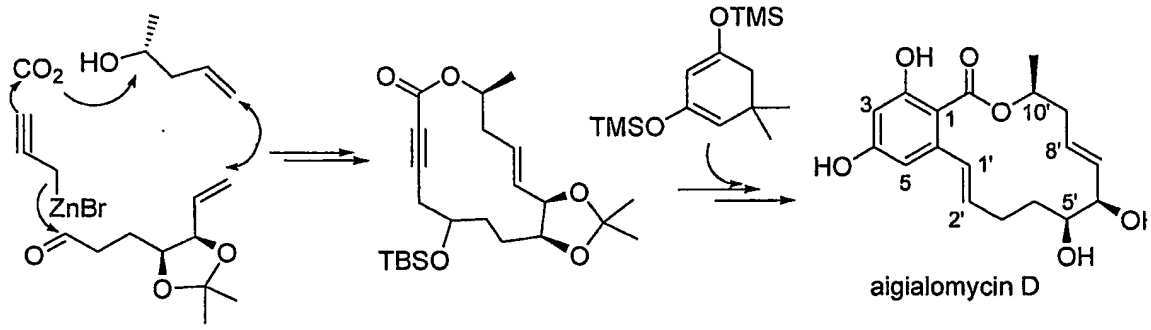
a) Synthesis of Radicicol and Monocillin:

As described in more detail below, a novel synthetic route to radicicol and monocillin has been developed, which methodology allows for the efficient generation of a variety of analogues of radicicol, monocillin, homodimers, heterodimers, and conjugates thereof.

The underlying general scheme for this new synthesis of radicicol is shows in the scheme below, which is directed to the synthesis of cycloproparadicicol. The central element of the synthesis is the production of an "ynolide" intermediate, which is prepared by olefin metathesis, and its reaction with a diene via a Diel-Alder cycloaddition to form the benzo-fused macrolide.



The same general approach has also been used to synthesize compounds of the invention including aigialomycin D as shown below.

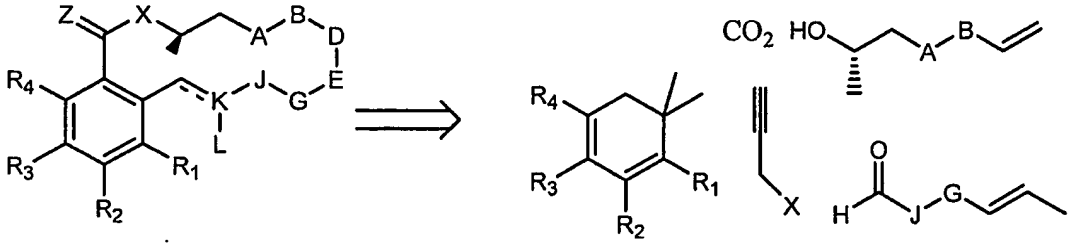


b) General Synthetic Methodology:

This new synthetic methodology provides for the rapid synthesis of a variety of analogues of radicicol, monocillin, dimers, and conjugates thereof. It will be appreciated that the ability to rapidly generate a range of analogues is important because it is believed that in vivo activity is lost due to certain structural characteristics of radicicol and monocillin. For example, it is postulated that in vivo activity is lost due to the nucleophilic action of cellular thiols (i.e. glutathione) on either the epoxide or the α,β unsaturated ketone of radicicol. This nucleophilic addition changes the overall conformation of radicicol, and results in an inability to bind to Hsp90. A second likely pathway of deactivation involves the conjugation of the aromatic ring, or perhaps cytochrome P-450 oxidation.

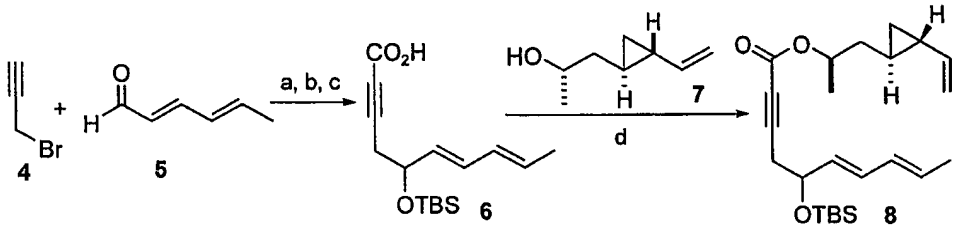
Without wishing to be bound by any particular theory, one strategy to restore in vivo activity would be to reduce the affinity of radicicol to nucleophiles such as thiols, specifically to deactivate electrophilic sites in radicicol. Here care must be taken not to dramatically change the overall conformation of the natural product. Thus these analogues have been designed to attenuate electrophilicity with simple alterations to the structure that should not affect the overall conformation. It should be emphasized that the analogues as described using the methodology herein cannot be made from the natural product. The three component nature of the synthetic process described above and described more generally below emphasizes the ability to generate numerous analogues by the modification of one component and its incorporation into a short and efficient process.

In general, the method involves the synthesis of analogues from four easily obtainable and diversifiable components. Depicted below is a general retrosynthetic strategy for the synthesis of analogues:



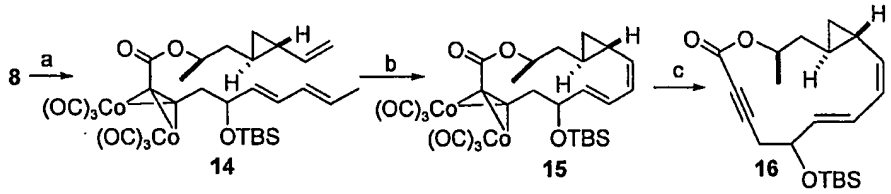
It should be noted that these analogs can be generated either with a single modification, or they may be combined in a single entity to maximize their benefit if they are found to be synergistic. It will also be appreciated, as described in more detail herein, that each of the components can be diversified prior to formation of the macrocycle, or alternatively or additionally, can be diversified after formation of the macrocycle.

As depicted generally below, the synthesis of analogues can be carried out in a similar fashion to the synthesis of radicicol and monocillin as described above. The synthesis of the acyclic alkynoic ester is shown below:



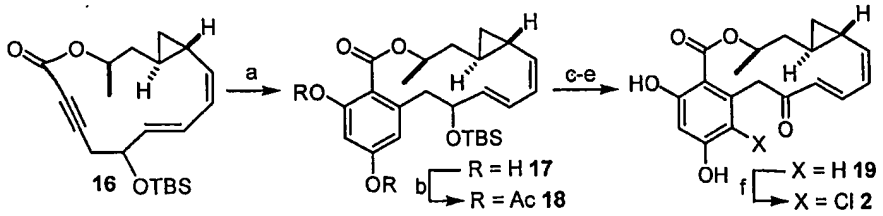
Reagents and conditions: (a) (i) Zn, THF, 66%; (b) TBSCl, imidazole, DMAP, CH_2Cl_2, 100%; (c) BuLi, -78 °C; then CO_2; (d) DIAD, Ph_3P, THF, -20 °C, 47% (two steps).

The resulting triene **8** is then treated with dicobalt carbonyl to complex with the acetylene. The combalt complex **14** is then cyclized under ring closing metathesis conditions to form **15**. The cobalt is then removed to give **16**.



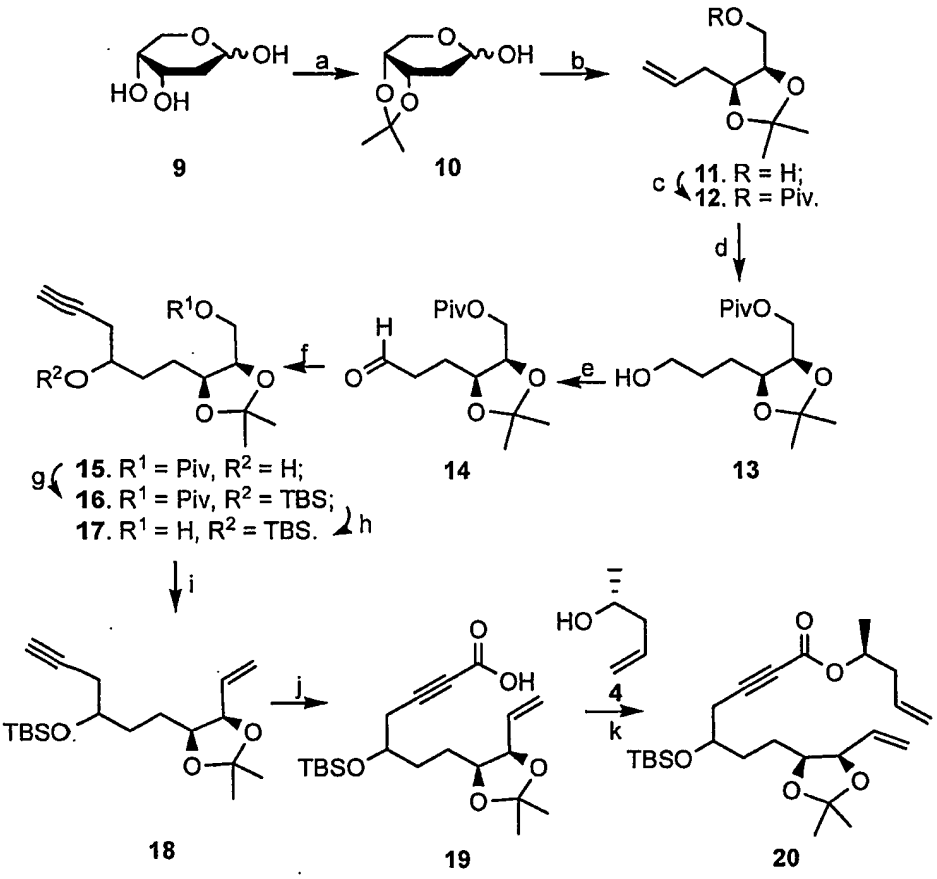
Reagents and conditions: (a) $Co_2(CO)_8$, PhMe, 100%; (b) 2nd genera-tion Grubbs catalyst (25 mol%), CH_2Cl_2 (0.2 mM), 45 °C, 57%; (c) I_2, THF, 0 °C, 69%.

The resulting ynolide **16** is then reacted with a diene using a Diels-Alder cycloaddition to yield **17**, which is then oxidized and chlorinated to give the desired product, cyclopropradicicol (**2**).



Reagents and conditions: (a) **12**, 140 °C, neat, 75%; (b) Ac_2O, DMAP, DMF, 87%; (c) HF/Pyr. THF; (d) Dess-Martin periodinane, CH_2Cl_2, 68% (two steps); (e) 5% $NaHCO_3$/MeOH, 92%; (f) SO_2Cl_2, CH_2Cl_2, 0 °C, 61%.

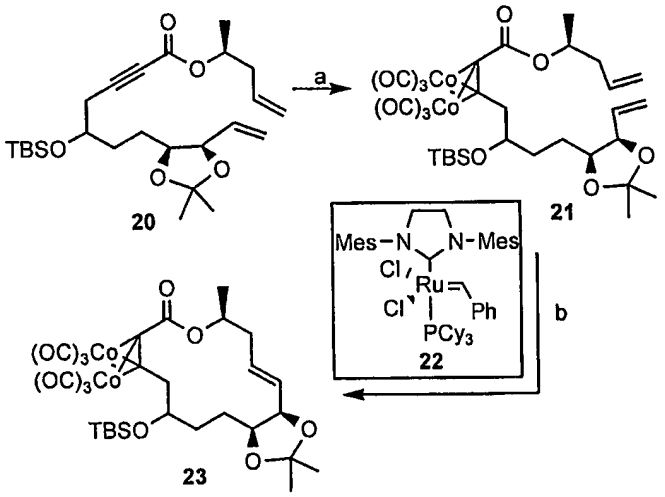
This basic approach can be used to prepare a variety of benofused macrolactones, including aigialomycin D. First the ring closing metathesis precurusor is made starting from D-2-deoxyribose.



a) 2-methoxypropene, *p*-TSA, DMF, 3 h, 62%; b) KHMDS, $Ph_3P^+CH_3I^-$, THF, -78 °C to r.t., 10 h, 68%; c) PivCl, Et_3N, DMAP, CH_2Cl_2, 10 h, 90%; d) 9-BBN, THF, 0 °C to r.t., 4 h, then NaOH, H_2O_2, H_2O, 2.5 h, 88%; e) SO_3-Pyr., DMSO, CH_2Cl_2, Et_3N, 0 °C, 1 h; f) propargyl bromide, zinc, THF, 0 °C, 2 h; g) TBSOTf, 2,6-lutidine, CH_2Cl_2, 10 h, 89% from **13**; h) NaOMe/MeOH, 10 h, 88%; i) SO_3-Pyr., DMSO, CH_2Cl_2, Et_3N, 0 °C,

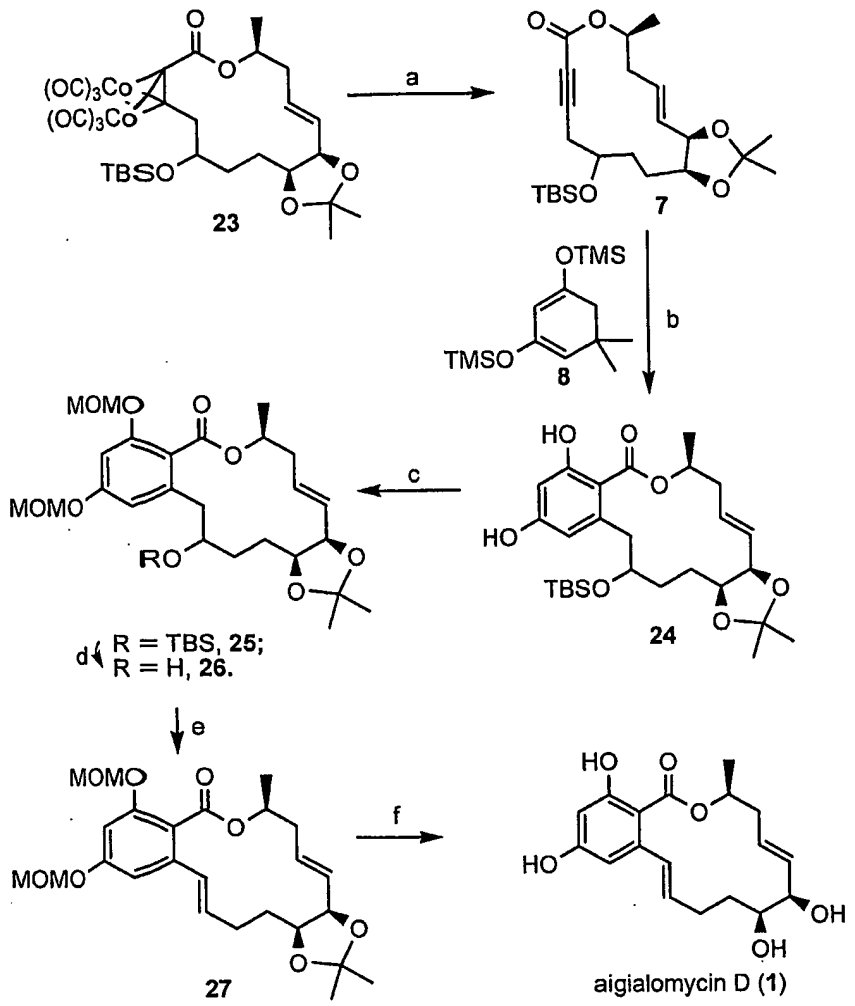
2 h, then KHMDS, $Ph_3P^+CH_3I^-$, THF, -78 °C to r.t., 10 h, 86% for two steps; j) BuLi, dry ice, -78 °C to r.t., 2 h; k) 4, DIAD, PPh_3, tol., 10 h, 85% for two steps.

The precursor is treated with dicobalt hexacarbonyl, and the resulting cobalt-complex is cyclized using a ring closing metathesis reaction.



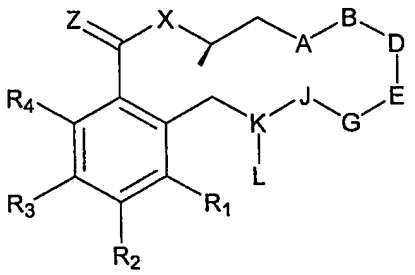
a) $Co_2(CO)_8$, tol., 30 min, 94%; b) 2nd generation Grubbs catalyst (25 mol%), CH_2Cl_2, 10 h, **23A**, 38%; **23B**, 42%.

The marocycle was then decomplexed and reacted with disiloxydiene to yield a benzo-fused macrocycle. The styrene double bond was installed, and the protecting groups were removed to yield aigialomycin D.



a). CAN, acetone, -10 °C, 15 min, **7A**, 94%; **7B** 95%; b). **8** neat, 140 °C, 36 h, **24A**, 74%; **24B**, 84%; c) MOMCl, DIPEA, CH_2Cl_2, 10 h, **25A**, 78%; **25B**, 83%; d) HF-pyr., pyr., THF, 10 h, **26A**, 78%; **26B**, 87%; e) $[PhC(CF_3)_2O]_2SPh_2$, CH_2Cl_2, 0 °C to r.t., 2 h, from **26A** to **27**, 90%; from **26B** to **27**, 84%; f) 0.5 N HCl, H_2O/MeOH, 2 d, 69%.

Thus, in addition to providing cycloproparadicicol as described above and herein, the present invention additionally provides a method for the synthesis of compounds having the general structure (I):



(I)

wherein

R_1 is hydrogen, halogen, an aliphatic, heteroaliphatic, aryl, heteroaryl, alkylaryl, or alkylheteroaryl moiety, or $N(R_A)_2$, wherein each occurrence of R_A is independently hydrogen, a protecting group, or an aliphatic, heteroaliphatic, aryl, heteroaryl, alkylaryl, or alkylheteroaryl moiety;

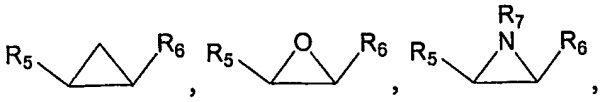
R_2 is hydrogen, halogen, cyano, $-OR_B$, $-N(R_B)_2$, $-SR_B$, $-O(C=O)R_B$, $-N(R_B)(C=O)(R_B)$, $-C(O)R_B$, $-C(O)OR_B$, $-CON(R_B)_2$, $-OCO_2R_B$, or an aliphatic, heteroaliphatic, aryl, heteroaryl, alkylaryl, or alkylheteroaryl moiety, wherein each occurrence of R_B is independently hydrogen, a protecting group or an aliphatic, heteroaliphatic, aryl, heteroaryl, alkylaryl, or alkylheteroaryl moiety;

R_3 is hydrogen, halogen, an aliphatic, heteroaliphatic, aryl, heteroaryl, alkylaryl, or alkylheteroaryl moiety, or $-N(R_C)_2$, wherein each occurrence of R_C is independently hydrogen, a protecting group, or an aliphatic, heteroaliphatic, aryl, heteroaryl, alkylaryl, or alkylheteroaryl moiety;

R_4 is hydrogen, halogen, cyano, $-OR_D$, $-N(R_D)_2$, $-SR_D$, $-O(C=O)R_D$, $-N(R_D)(C=O)(R_D)$, $-C(O)R_D$, $-C(O)OR_D$, $-CON(R_D)_2$, $-OCO_2R_D$, or an aliphatic, heteroaliphatic, aryl, heteroaryl, alkylaryl, or alkylheteroaryl moiety, wherein each occurrence of R_D is independently hydrogen, a protecting group or an aliphatic, heteroaliphatic, aryl, heteroaryl, alkylaryl, or alkylheteroaryl moiety;

Z is O, S or NR_E, wherein R_E is hydrogen, a protecting group, an aliphatic, heteroaliphatic, aryl, heteroaryl, alkylaryl, or alkylheteroaryl moiety, or OR_F, wherein R_F is hydrogen, a protecting group, an aliphatic, heteroaliphatic, aryl, heteroaryl, alkylaryl, or alkylheteroaryl moiety;

X is O, S or NR_G, wherein R_G is hydrogen or lower alkyl;

A and B together represent 

, -CHR_5-CHR_6-, -CR_5=CR_6-, wherein R_5 and R_6 are each independently hydrogen, halogen, cyano, -OR_J, -$N(R_J)_2$, -SR_J, -O(C=O)R_J, -O(S=O)R_J ,-$N(R_J)$(C=O)(R_J), -C(=O)R_J, -C(=O)OR_J,

-$CON(R_J)_2$, -OCO_2R_J, -OS(=O)OR_J or an aliphatic, heteroaliphatic, aryl, heteroaryl, alkylaryl, or alkylheteroaryl moiety, wherein each occurrence of R_J is independently hydrogen, a protecting group, or an aliphatic, heteroaliphatic, aryl, heteroaryl, alkylaryl, or alkylheteroaryl moiety, and wherein R_7 is hydrogen, a protecting group, -OR_K, -SR_K, -C(O)OR_K, -C(O)NR_K, -$S(O)_2R_K$, -O(C=O)R_K, -$N(R_K)$(C=O)(R_K), -C(O)R_K, -C(O)OR_K, -$CON(R_K)_2$, -OCO_2R_K, or an aliphatic, heteroaliphatic, aryl, heteroaryl, alkylaryl, or alkylheteroaryl moiety, wherein each occurrence of R_K is independently hydrogen, a protecting group or an aliphatic, heteroaliphatic, aryl, heteroaryl, alkylaryl, or alkylheteroaryl moiety, or when A and B together represent -CHR_5-CHR_6-, R_5 and R_6 taken together represent a substituted or unsubstituted 3-7 membered aliphatic, heteroaliphatic, aryl or heteroaryl ring,

D and E together represent -CHR_8-CHR_9-, -CR_8=CR_9-, wherein R_8 and R_9 are each independently hydrogen or lower alkyl;

G and J together represent -CHR_{10}-CHR_{11}-, -CR_{10}=CR_{11}-, wherein R_{10} and R_{11} are each independently hydrogen or lower alkyl;

K and L together represent C=O, C=S, CH-CH_3, CH-$CH(R_L)_2$, $C=C(R_L)_2$, -CH_2-, -C(-$S(CH_2)_3$S-)-, CH-OR_L, CH-SR_L, CH-$N(R_L)_2$, CH-$N(R_L)$(C=O)(R_L), C=N-O-R_L, CH-N=O, $C=C(R_L)$-$N(R_L)_2$, C=N-R_L, C=N-$N(R_L)_2$, or, if the dotted line --- represents a bond, whereby a double bond is present, then K and L together represent C-$N(R_L)_2$, wherein each occurrence of R_L is independently hydrogen, a protecting group, an aliphatic, heteroaliphatic, aryl, heteroaryl, alkylaryl, or alkylheteroaryl moiety, or two occurrences of R_L taken together represent a 3 to 7-membered cyclic aliphatic, heteroaliphatic, aromatic or heteroaromatic moiety;

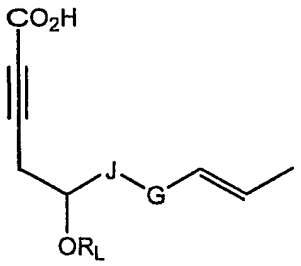
whereby each of the foregoing aliphatic and heteroaliphatic moieties may independently be substituted or unsubstituted, cyclic or acyclic, or branched or

unbranched, and each aryl, heteroaryl, alkylaryl, and alkylheteroaryl moiety may be substituted or unsubstituted;

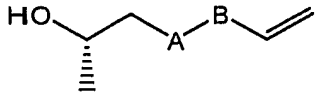
wherein one or any two of R_1, R_A, R_2, R_B, R_3, R_C, R_4, R_D, R_5, R_6, R_J, or R_L are optionally a linker covalently bonded to a compound selected from the group consisting of radicicol, monocillin, analogues of radicicol and monocillin, geldanamycin, analogues of geldanamycin, and steroids,

said method comprising:

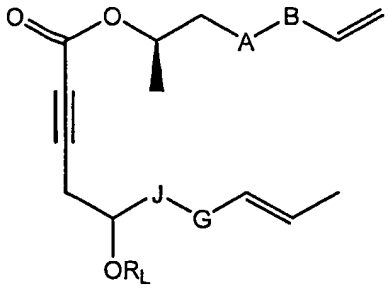
(1) reacting an acidic component having the structure:



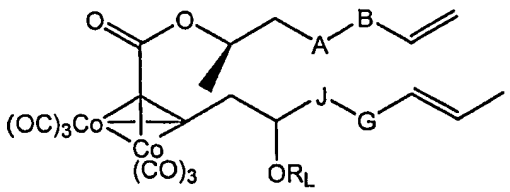
wherein R_L, J, and G are as defined above, with a chiral component having the structure:



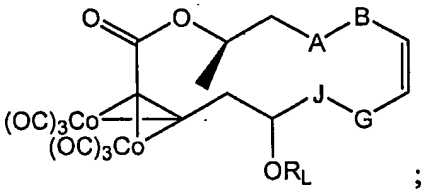
wherein A and B are as defined above, in the presence of an esterification reagent to generate an intermediate having the structure:



(2) complexing the intermediate with a cobalt, such as dicobalt hexcarbonyl, to yield a structure:



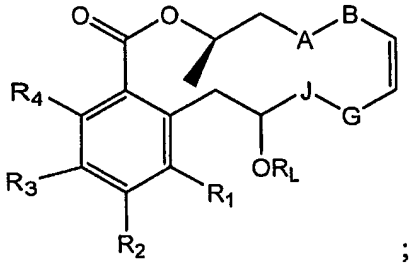
(3) cyclizing the combalt complex in the presence of an olefin metathesis catalyst to generate the compound:



;

(4) removing the cobalt to form a ynolide;

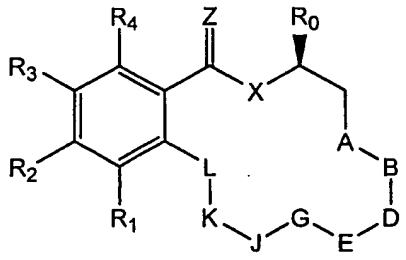
(5) reacting the alkyne moiety of the ynolide with a diene under cyclcoaddition conditions to generate the compound:



; and

(6) optionally further reacting the macrocycle with one or more reagents to diversify and optionally deprotecting the macrocycle to generate a compound having the formula (I).

Thus, in addition to providing aigialomycin D as described above and herein, the present invention additionally provides a method for the synthesis of compounds having the general structure (IIa):



(IIa)

wherein

R_0 is hydrogen, halogen, cyano, $-OR_Z$, $-N(R_Z)_2$, $-SR_Z$, $-O(C=O)R_Z$, $-N(R_Z)(C=O)(R_Z)$, $-C(O)R_Z$, $-C(O)OR_Z$, $-CON(R_Z)_2$, $-OCO_2R_Z$, or an aliphatic, heteroaliphatic, aryl, heteroaryl, alkylaryl, or alkylheteroaryl moiety, wherein each occurrence of R_Z is independently hydrogen, a protecting group, or an aliphatic, heteroaliphatic, aryl, heteroaryl, alkylaryl, or alkylheteroaryl moiety

R_1 is hydrogen, halogen, cyano, $-OR_A$, $-N(R_A)_2$, $-SR_A$, $-O(C=O)R_A$, $-N(R_A)(C=O)(R_A)$,
$-C(O)R_A$, $-C(O)OR_A$, $-CON(R_A)_2$, $-OCO_2R_A$, or an aliphatic, heteroaliphatic, aryl, heteroaryl, alkylaryl, or alkylheteroaryl moiety, wherein each occurrence of R_A is independently hydrogen, a protecting group, or an aliphatic, heteroaliphatic, aryl, heteroaryl, alkylaryl, or alkylheteroaryl moiety;

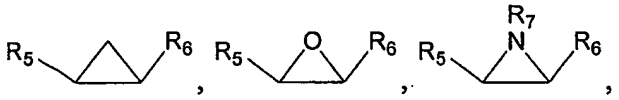
R_2 is hydrogen, halogen, cyano, $-OR_B$, $-N(R_B)_2$, $-SR_B$, $-O(C=O)R_B$, $-N(R_B)(C=O)(R_B)$,
$-C(O)R_B$, $-C(O)OR_B$, $-CON(R_B)_2$, $-OCO_2R_B$, or an aliphatic, heteroaliphatic, aryl, heteroaryl, alkylaryl, or alkylheteroaryl moiety, wherein each occurrence of R_B is independently hydrogen, a protecting group or an aliphatic, heteroaliphatic, aryl, heteroaryl, alkylaryl, or alkylheteroaryl moiety;

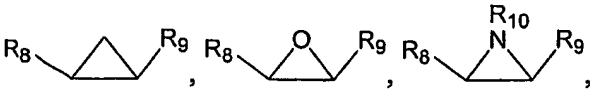
R_3 is hydrogen, halogen, cyano, $-OR_C$, $-N(R_C)_2$, $-SR_C$, $-O(C=O)R_C$, $-N(R_C)(C=O)(R_C)$,
$-C(O)R_C$, $-C(O)OR_C$, $-CON(R_C)_2$, $-OCO_2R_C$, or an aliphatic, heteroaliphatic, aryl, heteroaryl, alkylaryl, or alkylheteroaryl moiety, wherein each occurrence of R_C is independently hydrogen, a protecting group, or an aliphatic, heteroaliphatic, aryl, heteroaryl, alkylaryl, or alkylheteroaryl moiety;

R_4 is hydrogen, halogen, cyano, $-OR_D$, $-N(R_D)_2$, $-SR_D$, $-O(C=O)R_D$, $-N(R_D)(C=O)(R_D)$,
$-C(O)R_D$, $-C(O)OR_D$, $-CON(R_D)_2$, $-OCO_2R_D$, or an aliphatic, heteroaliphatic, aryl, heteroaryl, alkylaryl, or alkylheteroaryl moiety, wherein each occurrence of R_D is independently hydrogen, a protecting group or an aliphatic, heteroaliphatic, aryl, heteroaryl, alkylaryl, or alkylheteroaryl moiety;

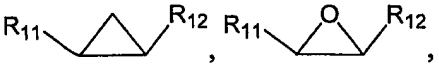
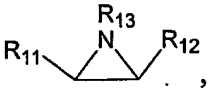
Z is O, S, or NR_E, wherein R_E is hydrogen, a protecting group, an aliphatic, heteroaliphatic, aryl, heteroaryl, alkylaryl, or alkylheteroaryl moiety, or OR_F, wherein R_F is hydrogen, a protecting group, an aliphatic, heteroaliphatic, aryl, heteroaryl, alkylaryl, or alkylheteroaryl moiety;

X is O, S or NR_G, wherein R_G is hydrogen or lower alkyl;

A and B together represent  , -CHR_5-CHR_6-, -CR_5=CR_6-, wherein R_5 and R_6 are each independently hydrogen, halogen, cyano, -OR_J, -$N(R_J)_2$, -SR_J, -O(C=O)R_J, -O(S=O)R_J ,-$N(R_J)$(C=O)(R_J), -C(=O)R_J, -C(=O)OR_J,
-$CON(R_J)_2$, -OCO_2R_J, -OS(=O)OR_J or an aliphatic, heteroaliphatic, aryl, heteroaryl, alkylaryl, or alkylheteroaryl moiety, wherein each occurrence of R_J is independently hydrogen, a protecting group, or an aliphatic, heteroaliphatic, aryl, heteroaryl, alkylaryl, or alkylheteroaryl moiety, and wherein R_7 is hydrogen, a protecting group, -OR_K, -SR_K, -C(O)OR_K, -C(O)NR_K, -$S(O)_2R_K$, -O(C=O)R_K, -$N(R_K)$(C=O)(R_K), -C(O)R_K, -C(O)OR_K, -$CON(R_K)_2$, -OCO_2R_K, or an aliphatic, heteroaliphatic, aryl, heteroaryl, alkylaryl, or alkylheteroaryl moiety, wherein each occurrence of R_K is independently hydrogen, a protecting group or an aliphatic, heteroaliphatic, aryl, heteroaryl, alkylaryl, or alkylheteroaryl moiety, or when A and B together represent -CHR_5-CHR_6-, R_5 and R_6 taken together represent a substituted or unsubstituted 3-7 membered aliphatic, heteroaliphatic, aryl or heteroaryl ring,

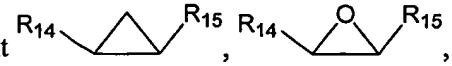
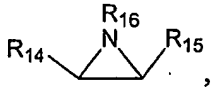
D and E together represent  , -CHR_8-CHR_9-, -CR_8=CR_9-, wherein R_8 and R_9 are each independently hydrogen, halogen, cyano, -OR_J, -$N(R_J)_2$, -SR_J, -O(C=O)R_J, -O(S=O)R_J ,-$N(R_J)$(C=O)(R_J), -C(=O)R_J, -C(=O)OR_J,
-$CON(R_J)_2$, -OCO_2R_J, -OS(=O)OR_J or an aliphatic, heteroaliphatic, aryl, heteroaryl, alkylaryl, or alkylheteroaryl moiety, wherein each occurrence of R_J is independently hydrogen, a protecting group, or an aliphatic, heteroaliphatic, aryl, heteroaryl, alkylaryl, or alkylheteroaryl moiety, and wherein R_{10} is hydrogen, a protecting group, -OR_K, -SR_K, -C(O)OR_K, -C(O)NR_K, -$S(O)_2R_K$, -O(C=O)R_K, -$N(R_K)$(C=O)(R_K), -C(O)R_K, -C(O)OR_K, -$CON(R_K)_2$, -OCO_2R_K, or an aliphatic, heteroaliphatic, aryl, heteroaryl,

alkylaryl, or alkylheteroaryl moiety, wherein each occurrence of R_K is independently hydrogen, a protecting group or an aliphatic, heteroaliphatic, aryl, heteroaryl, alkylaryl, or alkylheteroaryl moiety, or when A and B together represent $-CHR_8-CHR_9-$, R_9 and R_9 taken together represent a substituted or unsubstituted 3-7 membered aliphatic, heteroaliphatic, aryl or heteroaryl ring;

G and J together represent  ,  ,

$-CHR_{11}-CHR_{12}-$, $-CR_{11}=CR_{12}-$, wherein R_{11} and R_{12} are each independently hydrogen, halogen, cyano, $-OR_J$, $-N(R_J)_2$, $-SR_J$, $-O(C=O)R_J$, $-O(S=O)R_J$,$-N(R_J)(C=O)(R_J)$, $-C(=O)R_J$, $-C(=O)OR_J$,

$-CON(R_J)_2$, $-OCO_2R_J$, $-OS(=O)OR_J$ or an aliphatic, heteroaliphatic, aryl, heteroaryl, alkylaryl, or alkylheteroaryl moiety, wherein each occurrence of R_J is independently hydrogen, a protecting group, or an aliphatic, heteroaliphatic, aryl, heteroaryl, alkylaryl, or alkylheteroaryl moiety, and wherein R_{13} is hydrogen, a protecting group, $-OR_K$, $-SR_K$, $-C(O)OR_K$, $-C(O)NR_K$, $-S(O)_2R_K$, $-O(C=O)R_K$, $-N(R_K)(C=O)(R_K)$, $-C(O)R_K$, $-C(O)OR_K$, $-CON(R_K)_2$, $-OCO_2R_K$, or an aliphatic, heteroaliphatic, aryl, heteroaryl, alkylaryl, or alkylheteroaryl moiety, wherein each occurrence of R_K is independently hydrogen, a protecting group or an aliphatic, heteroaliphatic, aryl, heteroaryl, alkylaryl, or alkylheteroaryl moiety, or when A and B together represent $-CHR_{11}-CHR_{12}-$, R_{11} and R_{12} taken together represent a substituted or unsubstituted 3-7 membered aliphatic, heteroaliphatic, aryl or heteroaryl ring;

K and L together represent  ,  ,

$-CHR_{14}-CHR_{15}-$, $-CR_{14}=CR_{15}-$, wherein R_{14} and R_{15} are each independently hydrogen, halogen, cyano, $-OR_J$, $-N(R_J)_2$, $-SR_J$, $-O(C=O)R_J$, $-O(S=O)R_J$,$-N(R_J)(C=O)(R_J)$, $-C(=O)R_J$, $-C(=O)OR_J$,

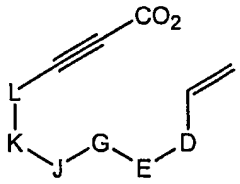
$-CON(R_J)_2$, $-OCO_2R_J$, $-OS(=O)OR_J$ or an aliphatic, heteroaliphatic, aryl, heteroaryl, alkylaryl, or alkylheteroaryl moiety, wherein each occurrence of R_J is independently hydrogen, a protecting group, or an aliphatic, heteroaliphatic, aryl, heteroaryl, alkylaryl,

or alkylheteroaryl moiety, and wherein R_{16} is hydrogen, a protecting group, $-OR_K$, $-SR_K$, $-C(O)OR_K$, $-C(O)NR_K$, $-S(O)_2R_K$, $-O(C=O)R_K$, $-N(R_K)(C=O)(R_K)$, $-C(O)R_K$, $-C(O)OR_K$, $-CON(R_K)_2$, $-OCO_2R_K$, or an aliphatic, heteroaliphatic, aryl, heteroaryl, alkylaryl, or alkylheteroaryl moiety, wherein each occurrence of R_K is independently hydrogen, a protecting group or an aliphatic, heteroaliphatic, aryl, heteroaryl, alkylaryl, or alkylheteroaryl moiety, or when A and B together represent $-CHR_{14}-CHR_{15}-$, R_{14} and R_{15} taken together represent a substituted or unsubstituted 3-7 membered aliphatic, heteroaliphatic, aryl or heteroaryl ring;

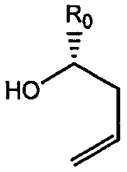
whereby each of the foregoing aliphatic and heteroaliphatic moieties may independently be substituted or unsubstituted, cyclic or acyclic, or branched or unbranched, and each aryl, heteroaryl, alkylaryl, and alkylheteroaryl moiety may be substituted or unsubstituted,

said method comprising:

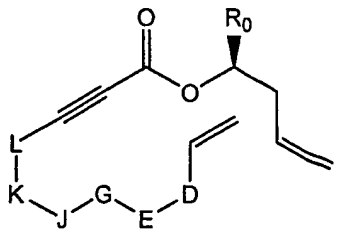
(1) reacting a component having the structure:



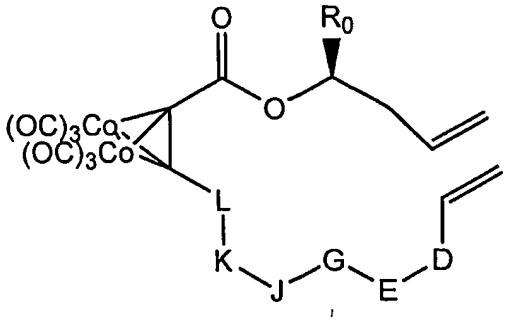
wherein R_L, J, and G are as defined above, with a chiral component having the structure:



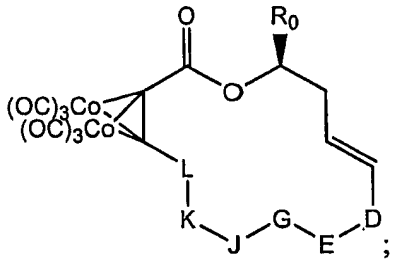
wherein A, B, D, E, G, J, K, and L are as defined above, in the presence of an esterification reagent to generate an intermediate having the structure:



(2) complexing the intermediate with a cobalt, such as dicobalt hexcarbonyl, to yield a structure:

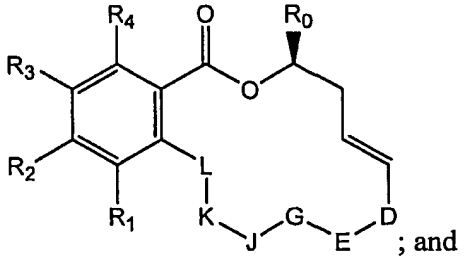


(3) cyclizing the combalt complex in the presence of an olefin metathesis catalyst to generate the compound:



(4) removing the cobalt to form a ynolide;

(5) reacting the alkyne moiety of the ynolide with a diene under cyclcoaddition conditions to generate the compound:



(6) optionally further reacting the macrocycle with one or more reagents to diversify and optionally deprotecting the macrocycle to generate a compound having the formula **(IIa)**.

In addition to the general method described and depicted above, the present invention provides additional synthetic methods, and compounds, as described herein, in which each of the intermediate steps and intermediate compounds are provided, as

described below and generically herein. It will be appreciated that the classes, and subclasses, as described above for the inventive compounds are also intended to encompass the inventive methods and intermediate compounds as described above and herein. Thus, certain classes and subclasses of interest in which the moieties R_0-R_4, Z, X, A-B, D-E, G-J and K-L are specifically defined (and moieties defined within those definitions) also apply to the inventive methods and intermediate compounds. It will be appreciated that certain exemplary species of the compounds of formula **(I)** and **(IIa)** and intermediates thereto are described herein, but are not limited to those species.

In certain embodiments, if any one or more of R_0-R_4 is a protected thio, amino or hydroxyl group, the method further comprises optionally deprotecting said unprotected group.

In still other embodiments, the method optionally further comprises reacting the intermediates with one or more reagents to diversify the macrocycle and generate a compound having the structure **(I)** or **(IIa)**. In one embodiment, the one or more positions on the benzene ring are halogenated (*e.g.*, chlorinated). In yet other embodiments, the method further comprises optionally deprotecting the compound having the structure **(I)** or **(IIa)**, to generate a deprotected compound having the structure **(I)** or **(IIa)**.

It will also be appreciated that each of the steps as described above can be carried out using reagents and conditions as described for the synthesis of radicicol or aigialomycins (as described below in the Examples), or they may be modified using other available reagents. For example, a variety of esterification conditions and olefin metathesis conditions are well-known in the art and can be utilized in the method of the invention. See, generally, March, *Advanced Organic Chemistry*, John Wiley & Sons, 1992.

As mentioned above, it will also be appreciated that each of the components used in the synthesis of analogues can be diversified either before synthesis or alternatively after the construction of the macrocycle. As used herein, the term "diversifying" or "diversify" means reacting an inventive compound or intermediate, as

defined herein, at one or more reactive sites to modify a functional moiety or to add a functional moiety. For example, the aromatic ring can be diversified to either add functionality (*e.g.*, where hydrogen is present, a halogen, *e.g.*, Cl, can be added) or to modify functionality (*e.g.*, where a hydroxyl group is present on the aromatic ring, the aromatic ring can be diversified by reacting with a reagent to protect the hydroxyl group, or in another example, by reacting with a reagent to add a linker moiety that has a conjugate (*e.g.*, geldanamycin, *etc.*) attached thereto). Described generally below are a variety of schemes to assist the reader in the synthesis of a variety of analogues, either by diversification of the intermediate components or by diversification of the macrocyclic structures.

The aromatic ring can incorporate numerous changes. Novel Diels Alder methodolgy can be utilized to present different substitution patterns around the benzene ring. It will be appreciated that traditional aromatic synthesis can also be utilized to access permutations on the aromatic core. For example, as shown in Figure 10, diversity can be generated at aromatic positions, in one embodiment, after generation of the core structure. Specifically, R_1 and R_3 include substitution patterns arising from a cross-coupling strategy as depicted to enable introduction of amino, aliphatic, heteroaliphatic, aryl, heteroaryl, and alkylheteroaryl moieties.

Another analog serves to replace the labile ester functionality with a stronger amide component. Esterase activity is responsible for the premature metabolism of many potential therapeutic agents, and therefore this analogs aims to prevent this activity. Simple modification of the chiral component and incorporation into this process provides a facile route to an amide analog.

As detailed above, a variety of reactions can be utilized to diversify the radicicol and/or monocillin and aigialomycin core structures either during assembly or after assembly of the macrocycle. An alternative strategy endeavours not only to restore in vivo activity and achieve diversity, but also to enhance it. In a fashion similar to that produced with geldanamycin, a number of known steroid hormones can linked to radicicol and analogs via an oxime technology that has already been shown to restore in vivo activity (*see* US Patent 6,239,168; US Patent 5,977,165; Soga *et al. Cancer Res.* **1999**, *59*, 2931-2938; each of which is incorporated herein by reference). The steroid

hormones bind specifically to receptors found in important Hsp90 complexes. In addition, dimers of radicicol or heterodimers of radicicol and geldanamycin will be used to probe the activity of bifunctional binding agents on Hsp90 activity (see Figure 15).

In addition to conjugation via oxime technology as described above and depicted in Figure 15, it will be appreciated that conjugation can be effected through available functionality on the aromatic ring or through the A-B moiety, as described generally herein. Figure 16 depicts a variety of methods that can be utilized to effect conjugation.

It will be appreciated that a variety of linkers can be utilized to effect conjugation of geldanamycin, radicicol, monocillin and analogues thereof and steroids to the inventive compounds. As described above, any one or any two of R_1, R_A, R_2, R_B, R_3, R_C, R_4, R_D, R_5, R_6, R_J, or R_L may potentially be a site for conjugation and conjugation can be effected through a linker covalently bonded to a compound to be conjugated. The term "linker" as used herein, is intended to encompass a chemical moiety that is capable of effecting a stable (e.g., sufficiently unhindered that the conjugation can be performed) covalent linkage between an inventive compound as described herein, and another conjugate (geldanamycin, radicicol, monocillin, analogues thereof as described herein and elsewhere) and steroids. It will be appreciated that a variety of linkers can be utilized, including, but not limited to heteroatom linkages (*e.g.*,
O-S(=O)O, -O-(C=O)-O-, heteroalkyl, *etc.*), and aliphatic or heteroaliphatic linkages, in which the aliphatic and heteroaliphatic linkages may be substituted or unsubstituted, branched or unbranched, or cyclic or acyclic. In certain embodiments for the compounds as described above, the linker is an aliphatic or heteroaliphatic moiety, whereby said aliphatic or heteroaliphatic moiety is substituted or unsubstituted, branched or unbranched, or cyclic or acyclic. For example, it will be appreciated that this linker may be of varying length, and that altering the length of the linker may, in certain circumstances, confer a therapeutic benefit. In general, the linker may be 1-12 carbon atoms in length, and may also be 1-10, 1-6, or 1-4 carbon atoms in length, in other embodiments of special interest. As described above, the linker may be a linear chain or a substituted chain, for example incorporating double or triple bonds, an aryl

group or a secondary or tertiary amine. In certain other embodiments for the compounds as described above, the linker is a moiety having one of the structures -$(CH_2)_n$-CH=CH-$(CH_2)_m$-, -$(CH_2)_p$-C≡C-$(CH_2)_q$-, or -$CH_2(CH_2)_sCH_2$-, wherein each occurrence of n, m, p, q and s is independently an integer from 0-10. As described generally above, it will be appreciated that one or more of the hydrogen atoms may be replaced with a substituent including, but not limited to alkyl, heteroalkyl, secondary or tertiary amine, hydroxyl, thiol, aryl, heteroaryl, alkylaryl, or alkylheteroaryl. Similar dimers, trimers, and conjugates and linkers used for the conjugation thereof are described in more detail in Kuduk *et al., Bioorg. Med. Chem. Lett.* **2000**, *10*, 1303-1306; Kuduk *et al. Bioorg. Med. Chem. Lett.*, **1999**, *9*, 1233-1238; Zheng *et al. Cancer Res.* **2000**, *60*, 2090-2094; and WO00/61578; each of which is incorporated herein by reference). Additional guidance for the preparation of conjugates can be found in US Patent 5,977,165 (which is incorporated herein by reference) (in which, for example, two radiciciol derivatives are linked via -O-S(=O)-O-).

In certain embodiments, the compound to be conjugated is selected from the group consisting of radicicol, monocillin, analogues of radicicol and monocillin, geldanamycin, analogues of geldanamycin, and steroids. In particular, any of the compounds of the present invention may be conjugated with one or two compounds of the same structure or with one or two compounds of different structures, such as geldanamycin, analogues thereof and steroids. The term "analogues", as used herein, is intended to encompass radiciciol analogues as described herein and elsewhere (*see, e.g.*, U.S. Patents 5,977,165, 5,731,343, and 5,597,846; each of which is incorporated herein by reference) and geldanamycin analogues generally described in the art (*see, e.g.*, WO00/61578; incorporated herein by reference). It will be appreciated that a variety of steroids can be utilized in the method of the present invention. In certain embodiments, steroids are utilized to develop selective cytotoxic agents directed towards cancer cells that express steroid receptors (*e.g.*, estrogen receptor). Suitable steroids for use in the present invention include, but are not limited to, estradiol, estradiol valerate, estradiol cypionate, ethinyl estradiol, mestranol, quinestrol, estrone, estrone sulfate, equilin, testosterone, androstenedione, dehydroepiandrosterone, estriol 16α-hydroxydehydro-epiandrosterone, and 16α-hydroxyandrostenedione, to name a few.

5) Uses, Formulation and Administration

Pharmaceutical Compositions

As discussed above this invention provides novel compounds which have biological properties which make them of interest for the treatment of cancer, in particular those cancers characterized in that they comprise Rb negative cancer cells. Accordingly, in another aspect of the present invention, pharmaceutical compositions are provided, which comprise any one of the compounds described herein (or a prodrug, pharmaceutically acceptable salt or other pharmaceutically acceptable derivative thereof), and optionally comprise a pharmaceutically acceptable carrier. In certain embodiments, these compositions optionally further comprise one or more additional therapeutic agents. Alternatively, a compound of this invention may be administered to a patient in need thereof in combination with the administration of one or more other therapeutic agents. For example, additional therapeutic agents for conjoint administration or inclusion in a pharmaceutical composition with a compound of this invention may be a cytotoxic agent or anticancer agent approved for the treatment of cancer, as discussed in more detail herein, or it may be any one of a number of agents undergoing approval in the Food and Drug Administration that ultimately obtain approval for the treatment of cancer (e.g., epothilones, geldanamycin, to name a few). It will also be appreciated that certain of the compounds of present invention can exist in free form for treatment, or where appropriate, as a pharmaceutically acceptable derivative thereof. According to the present invention, a pharmaceutically acceptable derivative includes, but is not limited to, pharmaceutically acceptable salts, esters, salts of such esters, or a prodrug or other adduct or derivative of a compound of this invention which upon administration to a patient in need is capable of providing, directly or indirectly, a compound as otherwise described herein, or a metabolite or residue thereof.

As used herein, the term "pharmaceutically acceptable salt" refers to those salts which are, within the scope of sound medical judgement, suitable for use in contact with the tissues of humans and lower animals without undue toxicity, irritation, allergic response and the like, and are commensurate with a reasonable benefit/risk ratio. Pharmaceutically acceptable salts of amines, carboxylic acids, and other types of

compounds, are well known in the art. For example, S. M. Berge, *et al.* describe pharmaceutically acceptable salts in detail in *J. Pharmaceutical Sciences*, *66*: 1-19 (1977), incorporated herein by reference. The salts can be prepared in situ during the final isolation and purification of the compounds of the invention, or separately by reacting the free base function with a suitable organic acid. Examples of pharmaceutically acceptable, nontoxic acid addition salts are salts of an amino group formed with inorganic acids such as hydrochloric acid, hydrobromic acid, phosphoric acid, sulfuric acid and perchloric acid or with organic acids such as acetic acid, oxalic acid, maleic acid, tartaric acid, citric acid, succinic acid or malonic acid or by using other methods used in the art such as ion exchange. Other pharmaceutically acceptable salts include adipate, alginate, ascorbate, aspartate, benzenesulfonate, benzoate, bisulfate, borate, butyrate, camphorate, camphorsulfonate, citrate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, formate, fumarate, glucoheptonate, glycerophosphate, gluconate, hernisulfate, heptanoate, hexanoate, hydroiodide, 2-hydroxy-ethanesulfonate, lactobionate, lactate, laurate, lauryl sulfate, malate, maleate, malonate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, nitrate, oleate, oxalate, palmitate, pamoate, pectinate, persulfate, 3-phenylpropionate, phosphate, picrate, pivalate, propionate, stearate, succinate, sulfate, tartrate, thiocyanate, p-toluenesulfonate, undecanoate, valerate salts, and the like. Representative alkali or alkaline earth metal salts include sodium, lithium, potassium, calcium, magnesium, and the like. Further pharmaceutically acceptable salts include, when appropriate, nontoxic ammonium, quaternary ammonium, and amine cations formed using counterions such as halide, hydroxide, carboxylate, sulfate, phosphate, nitrate, loweralkyl sulfonate and aryl sulfonate.

Additionally, as used herein, the term "pharmaceutically acceptable ester" refers to esters which hydrolyze in vivo and include those that break down readily in the human body to leave the parent compound or a salt thereof. Suitable ester groups include, for example, those derived from pharmaceutically acceptable aliphatic carboxylic acids, particularly alkanoic, alkenoic, cycloalkanoic and alkanedioic acids, in which each alkyl or alkenyl moiety advantageously has not more than 6 carbon atoms. Examples of particular esters includes formates, acetates, propionates, butyrates, acrylates and ethylsuccinates.

Furthermore, the term "pharmaceutically acceptable prodrugs" as used herein refers to those prodrugs of the compounds of the present invention which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of humans and lower animals with undue toxicity, irritation, allergic response, and the like, commensurate with a reasonable benefit/risk ratio, and effective for their intended use, as well as the zwitterionic forms, where possible, of the compounds of the invention. The term "prodrug" refers to compounds that are rapidly transformed in vivo to yield the parent compound of the above formula, for example by hydrolysis in blood. A thorough discussion is provided in T. Higuchi and V. Stella, Pro-drugs as Novel Delivery Systems, Vol. 14 of the A.C.S. Symposium Series, and in Edward B. Roche, ed., Bioreversible Carriers in Drug Design, American Pharmaceutical Association and Pergamon Press, 1987, both of which are incorporated herein by reference.

As described above, the pharmaceutical compositions of the present invention additionally comprise a pharmaceutically acceptable carrier, which, as used herein, includes any and all solvents, diluents, or other liquid vehicle, dispersion or suspension aids, surface active agents, isotonic agents, thickening or emulsifying agents, preservatives, solid binders, lubricants and the like, as suited to the particular dosage form desired. Remington's Pharmaceutical Sciences, Fifteenth Edition, E. W. Martin (Mack Publishing Co., Easton, Pa., 1975) discloses various carriers used in formulating pharmaceutical compositions and known techniques for the preparation thereof. Except insofar as any conventional carrier medium is incompatible with the anti-viral compounds of the invention, such as by producing any undesirable biological effect or otherwise interacting in a deleterious manner with any other component(s) of the pharmaceutical composition, its use is contemplated to be within the scope of this invention. Some examples of materials which can serve as pharmaceutically acceptable carriers include, but are not limited to, sugars such as lactose, glucose and sucrose; starches such as corn starch and potato starch; cellulose and its derivatives such as sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; powdered tragacanth; malt; gelatin; talc; excipients such as cocoa butter and suppository waxes; oils such as peanut oil, cottonseed oil; safflower oil; sesame oil; olive oil; corn oil and soybean oil; glycols; such a propylene glycol; esters such as ethyl oleate and ethyl laurate; agar; buffering agents such as magnesium hydroxide and aluminum hydroxide;

alginic acid; pyrogen-free water; isotonic saline; Ringer's solution; ethyl alcohol, and phosphate buffer solutions, as well as other non-toxic compatible lubricants such as sodium lauryl sulfate and magnesium stearate, as well as coloring agents, releasing agents, coating agents, sweetening, flavoring and perfuming agents, preservatives and antioxidants can also be present in the composition, according to the judgment of the formulator.

Uses of Compounds of the Invention

As described in more detail herein, in general, the present invention provides compounds useful for their ability to inhibit the growth of or kill cancer cells and thus are useful in the treatment of cancer. The compounds of the invention are also useful as inhibitors of Hsp90 and thus are useful, more generally as inhibitors of proteins such as transmembrane receptors (e.g., HER2, androgen receptor, erbB, EGFR, etc.), tyrosine kinases, serine and/or threonine kinases, transcriptional regulators, or of proteins that regulate them. In certain embodiments, the inventive compounds are also useful for the inhibition of the growth of or for the killing of Rb negative cancer cells and thus are useful in the treatment of cancers comprising Rb negative cancer cells. In certain other embodiments, the inventive compounds are also useful for the destruction of cells expressing a HER-family tyrosine kinase. In still other embodiments, the inventive compounds are useful as inhibitors of the androgen receptor.

In general, the unregulated growth characteristic of cancer cells typically results from disruption of a mitogenic signal transduction pathway. Such pathways can be disrupted at any of a number of points, through activation or inhibition of proteins such as transmembrane receptors (e.g., HER2, which is often overexpressed in breast cancers; steroid receptors such as the androgen receptor, which is often overexpressed in prostate cancers; erbB; EGFR; etc.), tyrosine kinases (including those that are domains of transmembrane receptors), serine and/or threonine kinases (e.g., Akt; Raf; Src; etc.), transcriptional regulators (e.g., Rb; STATs; etc.), or of proteins that regulate them. For instance, the molecular chaperone Hsp90 is required for proper folding of a variety of signal transduction proteins, including, for example, steroid receptors, HER2, met, Akt, Raf, etc. When Hsp90 activity is blocked, these proteins are degraded, and mitogenic signal transduction is attenuated.

It has been previously demonstrated that geldanamycin acts as an Hsp90 inhibitor; administration of this compound to tumor cells results in degradation of Hsp90-regulated proteins and arrest in the G1 phase of the cell cycle. Tumors that express high levels of HER2 are particularly sensitive to such agents. For example, treatment of tumors with geldamycin leads to a dose dependent reduction in HER2 levels as well as visible cellular differentiation. Unfortunately, the cell cycle arrest observed after treatment with these compounds is dependent upon the retinoblastoma (Rb) protein. Geldanamycin is currently in Phase II clinical trials, however, geldanamycin has been shown to be ineffective for the treatment of tumor cells with defective Rb function.

Unexpectedly, the present invention demonstrates that radicicol and radicicol analogs function as Hsp90 inhibitors independent of Rb function. Tumor cells treated with these compounds arrest in G1 in the presence of Rb, and arrest in the prometaphase stage of mitosis in the absence of Rb. Unlike geldanamycin, such compounds are therefore useful for the treatment of Rb-positive and Rb-negative cancers. Importantly, there are cancers which currently lack sufficient treatment, and are comprised of Rb negative cells. These include, but are not limited to, small-cell lung carcinoma, glioblastoma (brain) and retinoblastoma (eye). Small-cell lung carcinoma does not have an effective treatment, results in a high mortality rate, and represents 25% of lung cancers. In some embodiments, it may be desirable to combine administration of the inventive compounds described herein with proapoptotic chemotherapeutic agents and/or with radiation therapy in order to encourage arrested cells to enter apoptosis.

As demonstrated herein (see Figures 17-21), radicicol and certain of its analogues have been tested for their cytotoxicity in a panel of cancer cell lines, and importantly, for their ability to lead to the reduction of HER2. As depicted in Figure 17 and 18, four analogues have been tested for their ability to reduce HER2 in MCF7 and BT474 cells. Radicicol (**I**), monocillin I (**II**), cyclopropyl radicicol (**III**) and cyclopropyl monocillin I (**IV**) were each tested at similar concentrations (0.5 μM through 5 μM). These results demonstrate two previously unknown properties with respect to structure-activity relationships: the aromatic chloride contributes to efficacy, and the oxygen of the epoxide does not, despite its binding implication in the known

crystal structure. Radicicol is approximately 10x superior in effecting destruction of Her-2 than monocillin I, which only lacks the aromatic chloride. In addition, the cyclopropane analog (**III**) is at least as effective, if not better than radicicol merely by replacing the oxygen of the epoxide with a CH_2 group. Significantly, currently, the cyclopropane analog can only be made by the route as detailed herein. Furthermore, while the vinyl epoxide, a reactive moiety, may be responsible for undesired cytotoxicity, the cyclopropane is far more stabile to non-productive cellular nucleophiles.

Additionally, the dimethyl ethers of both radicicol and monocillin (**V** and **VI** respectively) have been tested, as well as the oxime disclosed by Kyowa Hakko (**VII**) in a side by side experiment with radicicol. Radicicol again demonstrated efficacy, as did the oxime analog **VII** which has been reported to also be active *in vivo*. As shown in Figure 19, MCF7 cells (HER2 overexpressed, Rb positive) were treated with radicicol (V-27), radicicol oxime (VI-51), dimethyl monocillin (V-25) and dimethyl radicicol (V-33) and the growth of the MCF7 cells was monitored.

As discussed above, the action of geldanamycin and radicicol on Hsp90 in Rb (retinoblastoma) positive cells results in a clear G_1 (growth) phase block of the cell cycle, degradation of HER2, and eventual reversion, apoptosis or necrosis. Interestingly, and without a clear explanation, when radicicol is applied to Rb negative cells, the cells are blocked in the M (mitosis) phase. While geldanamycin and its derivatives are demonstrating success in Rb positive cell lines, they are much less effective in their ability to halt growth in the corresponding Rb negative cells. Radicicol and derivatives appear to have an advantage in these Rb negative cell lines as they demonstrate superior efficacy blocking these cells in mitosis.

In addition, it has also been shown that 17-allylaminogeldanamycin (17-AGG), an ansamycin derivative, is much more potent against cells with wild type Rb than in cells with defective Rb function. It has also been shown that radicicol and cyclopropyl radicicol are potent inhibitors of both a breast cancer cell with wild type Rb and a small cell lung cancer cell line with defective Rb function (See Figure 20). Significantly, they have been found to be much more potent than 17-AGG in the latter cell type. These data suggest that radicicol derivatives are useful in the treatment of the 15-20% of

human tumors with mutated Rb gene and especially in small lung cell cancer, in which the gene is almost always mutated.

As discussed herein, the compounds of the present invention have been shown to reduce HER2 in MCF-7 and BT474 cells, and are cytotoxic against a panel of cancer cell lines (see exemplification herein and Figures 19, 20 and 21), in particular against Rb negative cancer cell lines (Figures 20 and 21). The inventive compounds are thus useful for the inhibition of the growth of or for killing cancer cells and are useful in the treatment of cancer (or more generally useful in the treatment of proliferative disorders). In addition, compounds as described herein have been found to act as potent inhibitors of cancer cell lines comprising Rb negative cells, and thus are useful in the treatment of cancers comprising Rb negative cells. Currently, there is no effective treatment for many of these cancers comprising Rb negative cells. The method of the invention comprises administering to a subject in need thereof a therapeutically effective amount of a compound of the invention.

In certain embodiments of the present invention a "therapeutically effective amount" of the inventive compound or pharmaceutical composition is that amount effective for detectable killing or inhibiting the growth of cancer cells, and in certain embodiments of special interest an amount for detectable killing or inhibiting the growth of cancer cells comprising Rb negative cancer cells.

The compounds and compositions, according to the method of the present invention, may be administered using any amount and any route of administration effective for killing or inhibiting the growth of tumor cells. Thus, the expression "effective amount" as used herein, refers to a sufficient amount of agent to kill or inhibit the growth of tumor cells. The exact amount required will vary from subject to subject, depending on the species, age, and general condition of the subject, the severity of the infection, the particular anticancer agent, its mode of administration, and the like. The anticancer compounds of the invention are preferably formulated in dosage unit form for ease of administration and uniformity of dosage. The expression "dosage unit form" as used herein refers to a physically discrete unit of anticancer agent appropriate for the patient to be treated. It will be understood, however, that the total daily usage of the compounds and compositions of the present invention will be decided by the attending physician within the scope of sound medical judgment. The specific therapeutically

effective dose level for any particular patient or organism will depend upon a variety of factors including the disorder being treated and the severity of the disorder; the activity of the specific compound employed; the specific composition employed; the age, body weight, general health, sex and diet of the patient; the time of administration, route of administration, and rate of excretion of the specific compound employed; the duration of the treatment; drugs used in combination or coincidental with the specific compound employed; and like factors well known in the medical arts.

Furthermore, after formulation with an appropriate pharmaceutically acceptable carrier in a desired dosage, the pharmaceutical compositions of this invention can be administered to humans and other animals orally, rectally, parenterally, intracisternally, intravaginally, intraperitoneally, topically (as by powders, ointments, or drops), bucally, as an oral or nasal spray, or the like, depending on the severity of the infection being treated. In certain embodiments, the compounds of the invention may be administered at dosage levels of about 0.001 mg/kg to about 50 mg/kg , from about 0.01 mg/kg to about 25 mg/kg, or from about 0.1 mg/kg to about 10 mg/kg of subject body weight per day, one or more times a day, to obtain the desired therapeutic effect. I will also be appreciated that dosages smaller than 0.001 mg/kg or greater than 50 mg/kg (for example 50-100 mg/kg) can be administered to a subject. In certain embodiments, compounds are administered orally or parenterally.

Liquid dosage forms for oral administration include, but are not limited to, pharmaceutically acceptable emulsions, microemulsions, solutions, suspensions, syrups and elixirs. In addition to the active compounds, the liquid dosage forms may contain inert diluents commonly used in the art such as, for example, water or other solvents, solubilizing agents and emulsifiers such as ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, 1,3-butylene glycol, dimethylformamide, oils (in particular, cottonseed, groundnut, corn, germ, olive, castor, and sesame oils), glycerol, tetrahydrofurfuryl alcohol, polyethylene glycols and fatty acid esters of sorbitan, and mixtures thereof. Besides inert diluents, the oral compositions can also include adjuvants such as wetting agents, emulsifying and suspending agents, sweetening, flavoring, and perfuming agents.

Injectable preparations, for example, sterile injectable aqueous or oleaginous suspensions may be formulated according to the known art using suitable dispersing or

wetting agents and suspending agents. The sterile injectable preparation may also be a sterile injectable solution, suspension or emulsion in a nontoxic parenterally acceptable diluent or solvent, for example, as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution, U.S.P. and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil can be employed including synthetic mono- or diglycerides. In addition, fatty acids such as oleic acid are used in the preparation of injectables.

The injectable formulations can be sterilized, for example, by filtration through a bacterial-retaining filter, or by incorporating sterilizing agents in the form of sterile solid compositions which can be dissolved or dispersed in sterile water or other sterile injectable medium prior to use.

In order to prolong the effect of a drug, it is often desirable to slow the absorption of the drug from subcutaneous or intramuscular injection. This may be accomplished by the use of a liquid suspension of crystalline or amorphous material with poor water solubility. The rate of absorption of the drug then depends upon its rate of dissolution which, in turn, may depend upon crystal size and crystalline form. Alternatively, delayed absorption of a parenterally administered drug form is accomplished by dissolving or suspending the drug in an oil vehicle. Injectable depot forms are made by forming microencapsule matrices of the drug in biodegradable polymers such as polylactide-polyglycolide. Depending upon the ratio of drug to polymer and the nature of the particular polymer employed, the rate of drug release can be controlled. Examples of other biodegradable polymers include poly(orthoesters) and poly(anhydrides). Depot injectable formulations are also prepared by entrapping the drug in liposomes or microemulsions which are compatible with body tissues.

Compositions for rectal or vaginal administration are preferably suppositories which can be prepared by mixing the compounds of this invention with suitable non-irritating excipients or carriers such as cocoa butter, polyethylene glycol or a suppository wax which are solid at ambient temperature but liquid at body temperature and therefore melt in the rectum or vaginal cavity and release the active compound.

Solid dosage forms for oral administration include capsules, tablets, pills, powders, and granules. In such solid dosage forms, the active compound is mixed with

at least one inert, pharmaceutically acceptable excipient or carrier such as sodium citrate or dicalcium phosphate and/or a) fillers or extenders such as starches, lactose, sucrose, glucose, mannitol, and silicic acid, b) binders such as, for example, carboxymethylcellulose, alginates, gelatin, polyvinylpyrrolidinone, sucrose, and acacia, c) humectants such as glycerol, d) disintegrating agents such as agar--agar, calcium carbonate, potato or tapioca starch, alginic acid, certain silicates, and sodium carbonate, e) solution retarding agents such as paraffin, f) absorption accelerators such as quaternary ammonium compounds, g) wetting agents such as, for example, cetyl alcohol and glycerol monostearate, h) absorbents such as kaolin and bentonite clay, and i) lubricants such as talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate, and mixtures thereof. In the case of capsules, tablets and pills, the dosage form may also comprise buffering agents.

Solid compositions of a similar type may also be employed as fillers in soft and hard-filled gelatin capsules using such excipients as lactose or milk sugar as well as high molecular weight polyethylene glycols and the like. The solid dosage forms of tablets, dragees, capsules, pills, and granules can be prepared with coatings and shells such as enteric coatings and other coatings well known in the pharmaceutical formulating art. They may optionally contain opacifying agents and can also be of a composition that they release the active ingredient(s) only, or preferentially, in a certain part of the intestinal tract, optionally, in a delayed manner. Examples of embedding compositions which can be used include polymeric substances and waxes. Solid compositions of a similar type may also be employed as fillers in soft and hard-filled gelatin capsules using such excipients as lactose or milk sugar as well as high molecular weight polethylene glycols and the like.

The active compounds can also be in micro-encapsulated form with one or more excipients as noted above. The solid dosage forms of tablets, dragees, capsules, pills, and granules can be prepared with coatings and shells such as enteric coatings, release controlling coatings and other coatings well known in the pharmaceutical formulating art. In such solid dosage forms the active compound may be admixed with at least one inert diluent such as sucrose, lactose or starch. Such dosage forms may also comprise, as is normal practice, additional substances other than inert diluents, e.g., tableting lubricants and other tableting aids such a magnesium stearate and microcrystalline

cellulose. In the case of capsules, tablets and pills, the dosage forms may also comprise buffering agents. They may optionally contain opacifying agents and can also be of a composition that they release the active ingredient(s) only, or preferentially, in a certain part of the intestinal tract, optionally, in a delayed manner. Examples of embedding compositions which can be used include polymeric substances and waxes.

Dosage forms for topical or transdermal administration of a compound of this invention include ointments, pastes, creams, lotions, gels, powders, solutions, sprays, inhalants or patches. The active component is admixed under sterile conditions with a pharmaceutically acceptable carrier and any needed preservatives or buffers as may be required. Ophthalmic formulation, ear drops, and eye drops are also contemplated as being within the scope of this invention. Additionally, the present invention contemplates the use of transdermal patches, which have the added advantage of providing controlled delivery of a compound to the body. Such dosage forms can be made by dissolving or dispensing the compound in the proper medium. Absorption enhancers can also be used to increase the flux of the compound across the skin. The rate can be controlled by either providing a rate controlling membrane or by dispersing the compound in a polymer matrix or gel.

As discussed above, in one aspect, the compounds of the present invention are useful as anticancer agents, and thus may be useful in the treatment of cancer, by effecting tumor cell death or inhibiting the growth of tumor cells. In general, the inventive anticancer agents are useful in the treatment of cancers and other proliferative disorders, including, but not limited to glioblastoma, retinoblastoms, breast cancer, cervical cancer, colon and rectal cancer, leukemia, lung cancer (including, but not limited to small cell lung cancer), melanoma, multiple myeloma, non-Hodgkin's lymphoma, ovarian cancer, pancreatic cancer, prostate cancer, and gastric cancer, to name a few. In certain embodiments, the inventive anticancer agents are active against cancers comprising Rb negative cells, including, but not limited to small cell lung cancer, retinoblastoma and glioblastoma. In certain other embodiments, the inventive anticancer agents are active against breast cancer cells, leukemia cells and melanoma cells, and thus are useful for the treatment of breast cancer, leukemias (e.g., myeloid, lymphocytic, myelocytic and lymphoblastic leukemias) and malignant melanomas. In

still other embodiments, the inventive anticancer agents are active against solid tumors and also kill and/or inhibit the growth of multidrug resistant cells (MDR cells).

It will also be appreciated that the compounds and pharmaceutical compositions of the present invention can be employed in combination therapies, that is, the compounds and pharmaceutical compositions can be administered concurrently with, prior to, or subsequent to, one or more other desired therapeutics or medical procedures. The particular combination of therapies (therapeutics or procedures) to employ in a combination regimen will take into account compatibility of the desired therapeutics and/or procedures and the desired therapeutic effect to be achieved. It will also be appreciated that the therapies employed may achieve a desired effect for the same disorder (for example, an inventive compound may be administered concurrently with another anticancer agent), or they may achieve different effects (e.g., control of any adverse effects).

For example, other therapies or anticancer agents that may be used in combination with the inventive anticancer agents of the present invention include surgery, radiotherapy (in but a few examples, γ-radiation, neutron beam radiotherapy, electron beam radiotherapy, proton therapy, brachytherapy, and systemic radioactive isotopes, to name a few), endocrine therapy, biologic response modifiers (interferons, interleukins, and tumor necrosis factor (TNF) to name a few), hyperthermia and cryotherapy, agents to attenuate any adverse effects (e.g., antiemetics), and other approved chemotherapeutic drugs, including, but not limited to, alkylating drugs (mechlorethamine, chlorambucil, Cyclophosphamide, Melphalan, Ifosfamide), antimetabolites (Methotrexate), purine antagonists and pyrimidine antagonists (6-Mercaptopurine, 5-Fluorouracil, Cytarabile, Gemcitabine), spindle poisons (Vinblastine, Vincristine, Vinorelbine, Paclitaxel), podophyllotoxins (Etoposide, Irinotecan, Topotecan), antibiotics (Doxorubicin, Bleomycin, Mitomycin), nitrosoureas (Carmustine, Lomustine), inorganic ions (Cisplatin, Carboplatin), enzymes (Asparaginase), and hormones (Tamoxifen, Leuprolide, Flutamide, and Megestrol), to name a few. Additionally, the present invention also encompasses the use of certain cytotoxic or anticancer agents currently in clinical trials and which may ultimately be approved by the FDA (including, but not limited to, epothilones and analogues thereof and geldanamycins and analogues thereof). For a more comprehensive discussion of

updated cancer therapies see, http://www.nci.nih.gov/, a list of the FDA approved oncology drugs at http://www.fda.gov/cder/cancer/druglistframe.htm, and The Merck Manual, Seventeenth Ed. 1999, the entire contents of which are hereby incorporated by reference.

TREATMENT KITS

In other embodiments, the present invention relates to a kit for conveniently and effectively carrying out the methods in accordance with the present invention. In general, the pharmaceutical pack or kit comprises one or more containers filled with one or more of the ingredients of the pharmaceutical compositions of the invention. Such kits are especially suited for the delivery of solid oral forms such as tablets or capsules. Such a kit preferably includes a number of unit dosages, and may also include a card having the dosages oriented in the order of their intended use. If desired, a memory aid can be provided, for example in the form of numbers, letters, or other markings or with a calendar insert, designating the days in the treatment schedule in which the dosages can be administered. Alternatively, placebo dosages, or calcium dietary supplements, either in a form similar to or distinct from the substituted purine dosages, can be included to provide a kit in which a dosage is taken every day. Optionally associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceutical products, which notice reflects approval by the agency of manufacture, use or sale for human administration.

EQUIVALENTS

The representative examples that follow are intended to help illustrate the invention, and are not intended to, nor should they be construed to, limit the scope of the invention. Indeed, various modifications of the invention and many further embodiments thereof, in addition to those shown and described herein, will become apparent to those skilled in the art from the full contents of this document, including the examples which follow and the references to the scientific and patent literature cited herein. It should further be appreciated that the contents of those cited references are incorporated herein by reference to help illustrate the state of the art.

The following examples contain important additional information, exemplification and guidance that can be adapted to the practice of this invention in its various embodiments and the equivalents thereof.

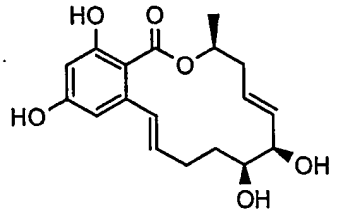
EXEMPLIFICATION

Example 1-Total Synthesis of Aigialomycin D

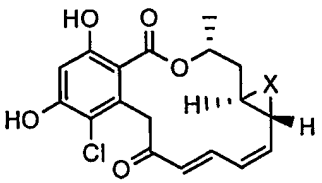
There are many natural products, usually bacterial metabolites, featuring a macrolide fused to a monocyclic benzenoid matrix, bearing a resorcinol-like substitution pattern. Not infrequently, the resorcinol moiety carries additional functionality, resulting in higher levels of oxidation. Natural products in this family (*cf. inter alia* radicicol (Delmotte, P.; Delmotte-Plaquee, J. *Nature* **1953**, *171*, 344; incorporated herein by reference), LL-Z-1640s (McGahren, W. J. *J. Org. Chem.* **1978**, *43*, 2339-2343; incorporated herein by reference), monocillins (Ayer, W. A.; Lee, S. P.; Tsuneda, A.; Hiratsuka, Y. *Can. J. Microb.* **1980**, *26*, 766-773; incorporated herein by reference), nordinone (Ayer, W. A.; Pena-Rodriguez, L. *Phytochemistry* **1987**, *26*, 1353-1355; incorporated herein by reference), and zearelenone (Sugawara, F.; Kim, K. W.; Kobayashi, K.; Uzawa, J.; Yoshida, S.; Murofushi, N.; Takahashi, N.; Strobel, G. A. *Phytochemistry* **1992**, *31*, 1987-1990; incorporated herein by reference) possess potentially exploitable patterns of antitumor, antibiotic and antimalarial activity. Indeed, we were first attracted to this structural series by radicicol (Delmotte, P.; Delmotte-Plaquee, J. *Nature* **1953**, *171*, 344; Sharma, S. V.; Agatsuma, T.; Nakano, H. *Oncogene* **1998**, *16*, 2639; each of which is incorporated herein by reference) – a non quinoidal inhibitor of the key molecular chaperone HSP90. Using radicicol (**2**) as a lead compound, we were soon led to cycloproparadicicol (**3**) (Yamamoto, K.; Garbaccio, R. M.; Stachel, S. J.; Solit, D. B.; Chiosis, G.; Rosen, N.; Danishefsky, S. J. *Angew. Chem., Int. Ed.* **2003**, *42*, 1280-1284; incorporated herein by reference) as a potentially valuable analog structure, wherein the cyclopropane simulates the conformational consequences of the epoxide, without the liabilities associated with a potentially labile alkylation site. Indeed, xenograft studies suggest that cycloproparadicicol (**3**) may well be a superior drug relative to radicicol (**2**). The

promise of cycloproparadicicol, albeit in an early preclinical setting, as well as the structural diversity encountered in this family of bioactive molecules, prompted us to explore new strategies for building such compounds in the laboratory. Indeed, a new strategy was described to reach cycloproparadicicol (Yang, Z.-Q., Danishefsky, S. J. *J. Am. Chem. Soc.* **2003**, *125*, 9602-9603; incorporated herein by reference).

Recently, five new 14-membered resorcyclic macrolides, termed aigailomycins A-E, were isolated from the marine mangrove fungus *Aigialus parvus* BCC5311 (Isaka, M.; Suyarnsestakorn, C.; Tanticharoen, M.; Kongsaeree, P.; Thebtaranonth, Y. *J. Org. Chem.* **2002**, *67*, 1561-1566; incorporated herein by reference). Among those compounds, aigialomycin D (**1**) (shown below) exhibited potent antimalarial activity (IC_{50}: 6.6 μg/mL against *P. falciparum*) and antitumor activity (IC_{50}: 3.0 μg/mL against KB cells) (Isaka, M.; Suyarnsestakorn, C.; Tanticharoen, M.; Kongsaeree, P.; Thebtaranonth, Y. *J. Org. Chem.* **2002**, *67*, 1561-1566; incorporated herein by reference).



aigialomycin D (**1**)



X = O, radicicol (**2**);
X = CH_2, cycloproparadicicol.

Resorcinylic macrolides: Aigialomycin D, radicicol, and cycloproparadicicol.

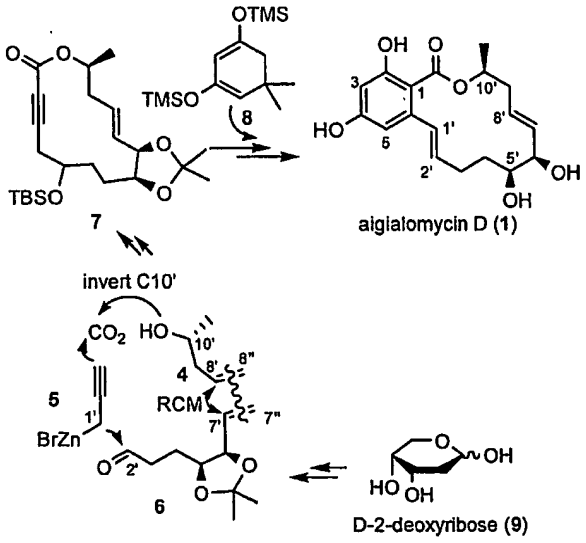
Not surprisingly, our first thought was to use the synthetic paradigm developed for cyclproparadicicol (Yang, Z.-Q., Danishefsky, S. J. *J. Am. Chem. Soc.* **2003**, *125*, 9602-9603; incorporated herein by reference). However, unlike **3**, **1** does not, in the end, contain benzylic oxygen functionality. Rather, it contains a 1', 2' styrene-like double bond, ortho to the acyloxyl group of the lactone. It was our plan to install this double bond by β-elimination of a C2' leaving group toward the benzo domain (*vide infra*). To bring about a pre-elimination setup, the initial bond formation would be between future carbons 1' and 2'. The more serious incremental complexity in the aigialomycin series arises from the two hdyroxy – bearing stereogenic centers at C5'

and C6' in allylic and homoallylic relationships respectively to the C7' – C8' double bond. The thought was to construct this double bond by ring forming olefin metatheses via extrusion of carbons 7" and 8". Scheme 1-1 (below) sets forth the thinking that led to a remarkably straightforward total synthesis of agialomycin D.

It was recognized that, if properly managed, the functionality present in the readily available D-2-deoxyribose (**9**) could lead to a functional equivalent of the key proposed formal building block **6**. Compound **6** would not be used as such (*vide infra*).

In the event, the secondary hydroxyl groups at C3 and C4 of **9**, were engaged in an isopropylidene linkage (see **10** (Barbat, J.; Gelas, J.; Horton, D. *Carbohydrate Res.* **1983**, *116*, 312-316; incorporated herein by reference), Scheme 1-2). The masked aldehyde character of C1 of the pentose could be exploited in the context of a Wittig protocol. The primary alcohol in the resultant product, **11**, was protected as its pivaloyl derivative (see **12**). In this compound, the primary methylene group bearing the pivaolyloxy group would emerge as C7' of the ring closing metathesis (RCM) precursor (*vide infra*). Hydroboration of **12** followed by oxidation, as shown, led us to **13** which, following oxidation of its primary alcohol function, delivered **14**.

Scheme 1-1. Synthetic Strategy.



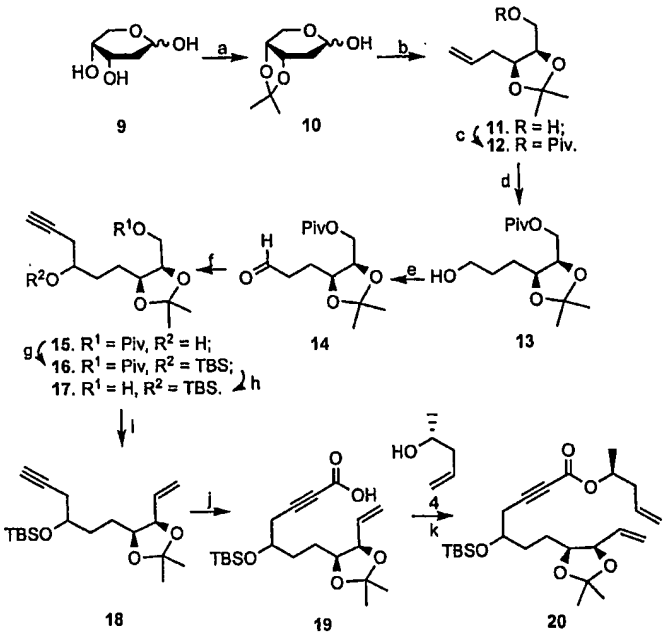
Chain extension of the aldehyde by propargylation afforded **15** as a mixture of

stereoisomers. In this compound, as well as in subsequent *seco* intermediates, these stereoisomers manifested nearly identical chromatographic characteristics. Thus, the mixtures were treated as single entities in the progression until compound **23**. The secondary hydroxyl groups in epimers **15** were protected as t-butyldimethyl silyl ethers (see **16**), thus enabling installation of a vinyl group, destined to serve as the C7'- C7" moiety in the eventual RCM (see steps leading to **18**).

Carboxylation of the ethynyl group in **18** occurred smoothly to afford carboxylic acid **19**. The latter reacted with *R* alcohol **4**, giving rise, through a Mitsunobu protocol, to the *S*-ester **20**, still bearing epimeric OTBS ethers at the future carbon **2'**.

Our previous experience (Yang, Z.-Q., Danishefsky, S. J. *J. Am. Chem. Soc.* **2003**, *125*, 9602-9603; incorporated herein by reference) had prepared us well for accomplishing the much needed ring closing metathesis reaction. First, it would be necessary to immobilize the ethynyl linkage in **20** as its dicobalt hexacarbonyl complex (Scheme 1-3) (Yang, Z.-Q., Danishefsky, S. J. *J. Am. Chem. Soc.* **2003**, *125*, 9602-9603; Nicholas, K. M.; Pettit, R. *Tetrahedron Lett.* **1971**, *37*, 3475-3478; Young, D. G. J.; Burlison, J. A.; Peters, U. *J. Org. Chem.* **2003**, *68*, 3494-3497; each of which is incorporated herein by reference). This step accomplishes two objectives. First, the acetylene function is insulated from diversion to an ene-yne metathesis format. Furthermore, it is likely that the formation of the complex modifies the angles of the ethynyl sector (Sternberg, H. W.; Greenfield, H.; Friedel, R. A.; Wotiz, J.; Markby, R.; Wender, I. *J. Am. Chem. Soc.* **1954**, *76*, 1457-1458; incorporated herein by reference), such as to bring carbons 7' and 8' into closer proximity. In the event, the hexacarbonyl complex **21** was obtained in 94% yield. Ring closing metathesis was easily accomplished, using the recently published catalysis methodology from the Grubbs group (Scholl, M.; Ding, S.; Lee, C. W.; Grubbs, R. H. *Org. Lett.* **1999**, *1*, 953-956; incorporated herein by reference). The 14 membered macrolide (**23**) was thus in hand. At this stage the two stereoisomeric products were easily separated by

Scheme 1-2. Synthesis of diene **20**.



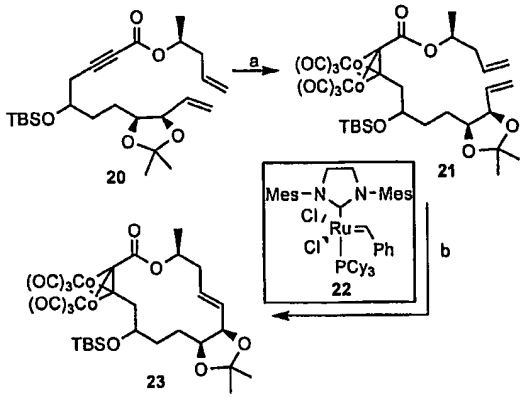
a) 2-methoxypropene, *p*-TSA, DMF, 3 h, 62%; b) KHMDS, $Ph_3P^+CH_3I^-$, THF, -78 °C to r.t., 10 h, 68%; c) PivCl, Et_3N, DMAP, CH_2Cl_2, 10 h, 90%; d) 9-BBN, THF, 0 °C to r.t., 4 h, then NaOH, H_2O_2, H_2O, 2.5 h, 88%; e) SO_3-Pyr., DMSO, CH_2Cl_2, Et_3N, 0 °C, 1 h; f) propargyl bromide, zinc, THF, 0 °C, 2 h; g) TBSOTf, 2,6-lutidine, CH_2Cl_2, 10 h, 89% from **13**; h) NaOMe/MeOH, 10 h, 88%; i) SO_3-Pyr., DMSO, CH_2Cl_2, Et_3N, 0 °C, 2 h, then KHMDS, $Ph_3P^+CH_3I^-$, THF, -78 °C to r.t., 10 h, 86% for two steps; j) BuLi, dry ice, -78 °C to r.t., 2 h; k) **4**, DIAD, PPh_3, tol., 10 h, 85% for two steps.

chromatography to provide the individual compound(s) at a 1.2:1 ratio (stereochemistry not rigorously assigned). We note that in each diastereomer, only the *E* configured double bond was obtained (J ≅ 15.2 Hz).

Decomplexation of the two separated compounds **23a** and **23b**, using standard conditions, thereby afforded "ynolide" epimers **7** (Scheme 4). Each stereoisomer was subjected to Diels Alder reaction with the disiloxydiene **8**, following previously developed conditions (Yang, Z.-Q., Danishefsky, S. J. *J. Am. Chem. Soc.* **2003**, *125*, 9602-9603; incorporated herein by reference). In the event, cycloaddition followed by extrusion of isobutylene occurred smoothly affording macrolides **24a** and **24b**. It proved useful to protect the two resorcycilic hydroxyl groups in the form of their MOM derivatives (see **25a** and **25b**) before proceeding with installation of the styrene like double bond. At this stage, deprotection of the silyl group was accomplished through the agency of HF-pyridine. Indeed, dehydration of the resulting alcohol functions in

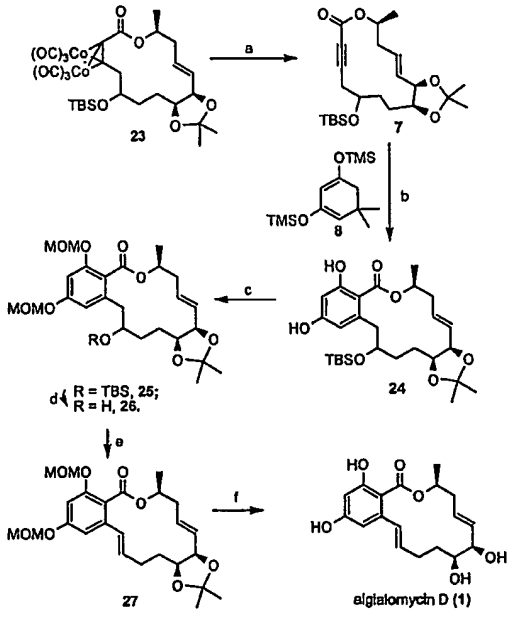
26a and **26b**, each using Martin's sulfurane conditions (Martin, J. C.; Arhart, R. J. *J. Am. Chem. Soc.* **1971**, *93*, 4327-4329; incorporated herein by reference), resulted in installation of the C1'-C2' double

Scheme 1-3. Synthesis of macrolactone **23** through RCM.



a) $Co_2(CO)_8$, tol., 30 min, 94%; b) 2nd generation Grubbs catalyst (25 mol%), CH_2Cl_2, 10 h, **23A**, 38%; **23B**, 42%.

Scheme 1-4. Completion of the total synthesis of aigialomycin D.

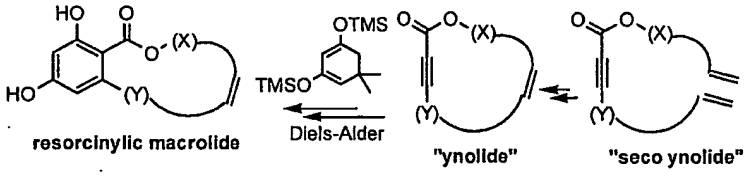


a). CAN, acetone, -10 °C, 15 min, **7A**, 94%; **7B** 95%; b). **8** neat, 140 °C, 36 h, **24A**, 74%; **24B**, 84%; c) MOMCl, DIPEA, CH_2Cl_2, 10 h, **25A**, 78%; **25**B, 83%; d) HF-pyr., pyr., THF, 10 h, **26A**, 78%; **26B**, 87%; e) $[PhC(CF_3)_2O]_2SPh_2$, CH_2Cl_2, 0 °C to r.t., 2 h, from **26A** to **27**, 90%; from **26B** to **27**, 84%; f) 0.5 N HCl, H_2O/MeOH, 2 d, 69%.

bond with the formation of the identical product, **27**. Global acidic deprotection of the two MOM functions and the acetonide (0.5 N HCl) served to complete the first total synthesis of aigialomycin D (**1**). The assignment of structural and relative configuration could well have been rigorously accomplished based on our measurements (proton NMR, carbon NMR, mass spec and IR) accumulated on the fully synthetic material. In the case at hand, further support comes from the very close correspondence of our data with those previously reported for the target structure aigialomycin D (Isaka, M.; Suyarnsestakorn, C.; Tanticharoen, M.; Kongsaeree, P.; Thebtaranonth, Y. *J. Org. Chem.* **2002**, *67*, 1561-1566; incorporated herein by reference).

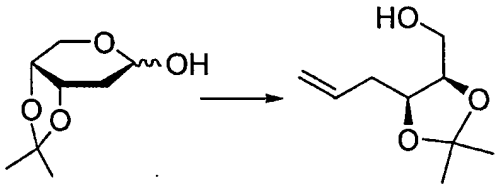
The synthesis described above, serves to further demonstrate the adaptability and generalizability of the basic protocol ("seco ylolide" → "ylolide" → resorcinylic macrolide, see Scheme 1-5). The total synthesis of **1** was accomplished in 18 steps in an overall yield of approximately 8%. We note in passing that compound **1** does bind to HSP90 (though significantly less so than radicicol (**2**)). It will be interesting to attempt to utilize this newly acquired and highly concise route to matrices resembling radicicol for the purposes of discovering new and superior agents based on the theme of HSP90 inhibition.

Scheme 1-5. "Ynolide" synthetic protocol.



Experimentals:

General Methods: Reagents obtained from commercial suppliers were used without further purification unless otherwise noted. THF, toluene, and methylene chloride was obtained from a dry solvent system (passed through a prepacked column of alumina) and used without further drying. All air and water sensitive reactions were performed in flame-dried glassware under a positive pressure of prepurified argon gas. NMR (^1H and ^{13}C) spectra were recorded on Bruker AMX-400 MHz or Bruker Advance DRX-500 MHz as noted individually, referenced to $CDCl_3$ (7.27 ppm for ^1H and 77.0 ppm for ^{13}C) or CD_3COCD_3 (2.09 ppm for ^1H and 30.6 and 205.9 ppm for ^{13}C). Infrared spectra (IR) were obtained on a Perkin-Elmer FT-IR model 1600 spectrometer. Melting point was tested on a electrothermal series IA9100 digital melting point apparatus. Optical rotations were obtained on a JASCO model DIP-370 digital polarimeter. Analytical thin-layer chromatography was performed on E. Merck silica gel 60 F254 plates. Compounds which were not UV active were visualized by dipping the plates in para-anisaldehyde solution and heating. Preparative thin layer chromatography was performed using the indicated solvent on Whatman® (LK6F Silica gel 60 Å 250 μM or Pk6F Silica Gel 60 Å 1000 μM) TLC plate.

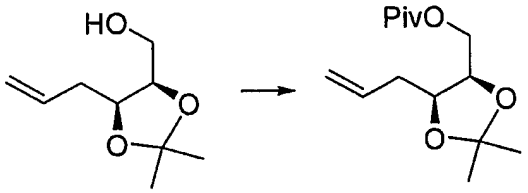


10 11

(*2R, 3S*)-Hex-5-ene-1,2,3-triol 2,3-acetonide (11):

To a stirring suspension of $Ph_3P^+CH_3I^-$ (11.2 g, 27.7 mmol) in 30 mL THF was added KHMDS (0.5 M in toluene, 46.0 mL, 23.0 mmol) at -78 °C. The solution was warmed up to 0 °C and stirred for 30 min before cooled down to -78 °C. Acetonide **10** (Barbat, J.; Gelas, J.; Horton, D. *Carbohydrate Res.* **1983**, *116*, 312-316; incorporated herein by reference) (1.6 g, 9.2 mmol) in 5 mL THF was added via cannula and the solution was warmed up to r.t. overnight (10 h) before quenched with saturated aqueous NH_4Cl solution, extracted with EtOAC (100 mL X 3). The organic layers were combined and dried with anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure vacuum. The residue was purified on a silica gel column using petroleum ether/EtOAc

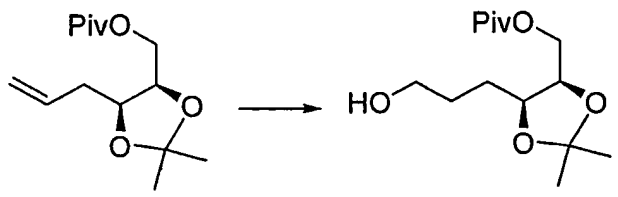
(4/1) as the eluant to afford **11** as a colorless oil (1.02 g, 68%). $[\alpha]_D^{25}$ 54.8 (*c* 0.26, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) δ 1.34 (s, 3 H), 1.46 (s, 3 H), 2.02 (b, 1 H), 2.25-2.32 (m, 1 H), 2.37-2.44 (m, 1 H), 3.65 (m, 1 H), 4.16-4.21 (m, 1 H), 4.24-4.28 (m, 1 H), 5.10-5.18 (m, 2 H), 5.79-5.89 (m, 1 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 25.4, 28.1, 33.6, 61.6, 76.2, 77.8, 108.3, 117.3, 134.2. LRMS (ESI) calcd for $C_9H_{16}O_3Na^+$ $[M+Na]^+$: 195.1, found 194.9. LRMS (ESI) calcd for $C_9H_{16}O_3Cl^-$ $[M+Cl]^-$: 207.1, found 207.1.



11 **12**

Pivalate (12):

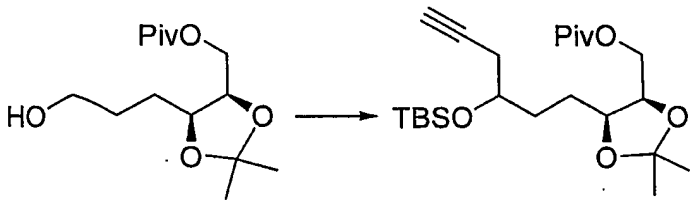
To a solution of **11** (752 mg, 4.37 mmol), DMAP (106 mg, 0.874 mmol) and triethylamine (2.5 ml, 17.5 mmol) in CH_2Cl_2 (8 ml) was added PivCl (1.1 ml, 8.74 mmol) at 0 °C. The reaction mixture was warmed up to r.t. overnight (10h) before quenched with saturated aqueous $NaHCO_3$ solution, extracted with EtOAC (100 mL X 3). The organic layers were combined and dried with anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure vacuum. The residue was purified on a silica gel column using hexanes/EtOAc (4/1) as the eluant to afford **12** as a colorless oil (984 mg, 90%). $[\alpha]_D^{25}$ 15.6 (*c* 0.32, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) δ 1.22 (s, 9 H), 1.33 (s, 3 H), 1.47 (s, 3 H), 2.28-2.41 (m, 2 H), 4.10-4.13 (m, 2 H), 4.22-4.29 (m, 2 H), 5.10-5.17 (m, 2 H), 5.81-5.91 (m, 1 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 25.9, 27.5, 28.4, 39.1, 63.2, 75.5, 77.8, 108.8, 117.7, 134.6, 178.5. LRMS (ESI) calcd for $C_{14}H_{24}O_4Na^+$ $[M+Na]^+$ 279.2, found 278.9.



12 **13**

Alcohol 13:

To a solution of **12** (166 mg, 0.648 mmol) in THF (1.5 mL) was added 9-BBN (0.5 M in THF, 2.8 mL, 1.425 mmol) at 0 °C. The reaction mixture was warmed up to r.t. over 4 h and H_2O (0.1 mL), NaOH (3 M, 0.7 mL) and H_2O_2 (30%, 0.2 mL) were added. The reaction mixture was diluted with H_2O 2.5 h later and acidified with citric acid (5%) till pH = 7. The mixture was extracted with EtOAc, washed with saturated aqueous $Na_2S_2O_3$, H_2O and brine. The organic layers were combined and dried with anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure vacuum. The residue was purified on a silica gel column using petroleum ether /EtOAc (1/1) as the eluant to afford **13** as a colorless oil (156 mg, 88%). $[\alpha]_D^{25}$ 82.4 (*c* 0.07, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) δ 1.21 (s, 9 H), 1.36 (s, 3 H), 1.46 (s, 3 H), 1.59-1.80 (m, 4 H), 2.30 (b, 1 H), 3.54 (m, 2 H), 4.08 (dd, *J* = 6.1, 11.5 Hz, 1 H), 4.12 (dd, *J* = 5.6, 11.5 Hz, 1 H), 4.19 (m, 1 H), 4.25 (m, 1 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 25.5, 25.9, 27.1, 28.0, 29.9, 38.7, 62.3, 62.9, 75.3, 77.0, 108.3, 178.2. HRMS (ESI) calcd for $C_{14}H_{26}O_5Na^+$ $[M+Na]^+$ 297.1678, found 297.1660, Δ = -6.0 ppm.



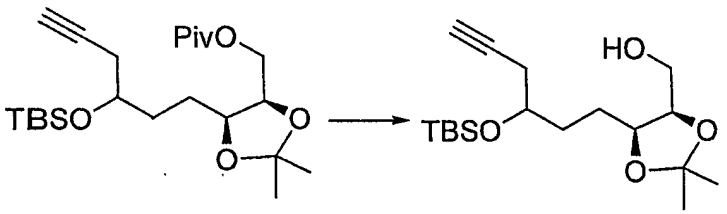
13 **16**

Alkyne 16:

To a solution of **13** (682 mg, 2.485 mmol) in CH_2Cl_2 (5 mL), DMSO (5 mL) and Et_3N (3 mL) was added SO_3-pyridine complex (1.6 g, 10.2 mmol) at 0 °C. The reaction mixture was stirred for 1 h before diluted with EtOAc and washed with HCl (0.5 *N*), H_2O, saturated aqueous $NaHCO_3$ solution and brine. The organic layers were dried with anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure vacuum. The crude aldehyde **14** was used directly for next step without any further purification.

To a suspension of Zn (Nano-size power, pre-activated, 390 mg, 5.949 mmol) in THF (10 mL) was added propargyl bromide (80% in toluene, 0.53 mL, 4.759 mmol) at 0 °C. The reaction mixture was stirred for 1 h at 0 °C and a solution of aldehyde **14** thus obtained in THF (5 mL) was added and reaction mixture was warmed up to r.t.

over 2 h before quenched with saturated aqueous NH_4Cl solution and extracted with EtOAc. The organic layers were dried with anhydrous $MgSO_4$, filtered, and concentrated under vacuum. To a solution of crude alcohol **15** thus obtained and 2,6-lutidine (0.6 mL, 4.76 mmol) in CH_2Cl_2 (8 mL) was added TBSOTf (0.82 mL, 3.57 mmol) and the reaction mixture was stirred for 10 h before quenched with saturated aqueous NH_4Cl solution, extracted with EtOAc (100 mL X 3). The organic layers were combined and dried with anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure vacuum. The residue was purified on a silica gel column using petroleum ether/EtOAc (20/1) as the eluant to afford **16** as a colorless oil (896 mg, 89% from **13**). ^1H NMR (400 MHz, $CDCl_3$) δ -0.02-0.05 (m, 6 H), 0.80 (s, 9 H), 1.13 (s, 9 H), 1.26 (s, 3 H), 1.36 (s, 3 H), 1.42-1.74 (m, 4 H), 1.81 (m, 1 H), 2.22-2.29 (m, 2 H), 3.78 (m, 1 H), 4.01-4.08 (m, 3 H), 4.12-4.17 (m, 1 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 18.0, 18.1, 24.3, 24.7, 25.6, 25.8, 27.0, 27.1, 27.2, 28.06, 28.07, 33.2, 33.3, 38.7, 62.8, 62.9, 70.1, 70.2, 70.4, 70.5, 75.2, 76.1, 77.1, 81.2, 108.20, 108.24, 178.1. HRMS (FAB) calcd for $C_{23}H_{42}O_5SiH^+$ $[M+H]^+$: 427.2880, found 427.2880, Δ = -0.1 ppm.

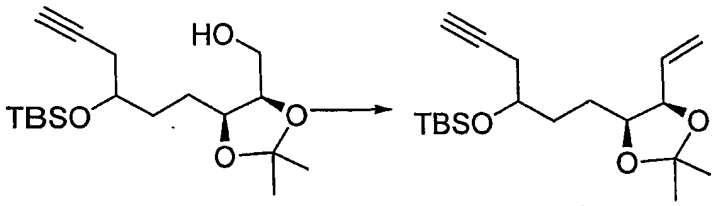


16 **17**

Alcohol 17:

To a solution of **16** (124 mg, 0.291 mmol) in MeOH (6 mL) was added NaOMe/MeOH (25%, 0.2 mL) and the reaction mixture was stirred for 10 h before quenched with saturated aqueous NH_4Cl solution, extracted with EtOAc (100 mL X 3). The organic layers were combined and dried with anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure vacuum. The residue was purified on a silica gel column using petroleum ether/EtOAc (8/1) as the eluant to afford **17** as a colorless oil (87 mg, 88%). ^1H NMR (400 MHz, $CDCl_3$) δ -0.02-0.00 (m, 6 H), 0.80 (s, 9 H), 1.32 (s, 3 H), 1.38 (s, 3 H), 1.32-1.91 (m, 4 H), 1.90 (m, 1 H), 1.96 (b, 1 H), 2.23-2.31 (m, 2 H), 3.53 (m, 2 H), 3.75 (m, 1 H), 4.08 (m, 1 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ -4.7, -4.6, -4.5, 18.0,

24.5, 24.7, 25.5, 25.6, 25.8, 27.2, 27.4, 28.2, 33.2, 33.4, 61.7, 70.1, 70.2, 70.46, 70.52, 76.9, 77.0, 77.9, 81.16, 81.24, 108.08, 108.14. HRMS (FAB) calcd for $C_{18}H_{34}O_4SiH^+$ $[M+H]^+$: 343.2305, found 343.2305, Δ = -0.1 ppm.



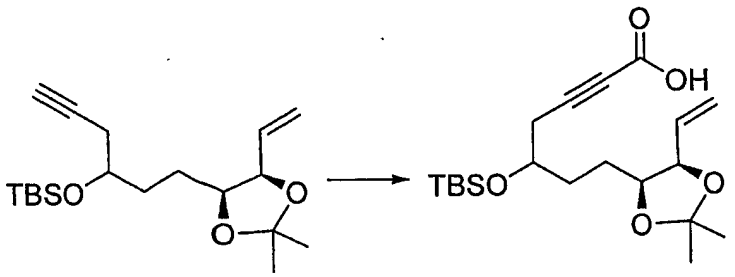
17 18

Enyne 18:

To a solution of **17** (87 mg, 0.254 mmol) in DMSO (1.0 mL), CH_2Cl_2 (1.0 mL) and Et_3N (1.0 mL) was added SO_3-Pyrdine complex (200 mg, 2.032 mmol) at 0 °C. The reaction mixture was stirred for 2 h before diluted with EtOAc and washed with HCl (0.5 *N*), H_2O, saturated aqueous $NaHCO_3$ solution and brine. The organic layers were dried with anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure vacuum. The crude aldehyde was used directly for next step without any further purification.

To a stirring suspension of $Ph_3P^+CH_3I^-$ (204 mg, 0.505 mmol) in 3 mL THF was added KHMDS (0.5 M in toluene, 0.9 mL, 0.454 mmol) at -78 °C. The solution was warmed up to 0 °C and stirred for 30 min before cooled down to -78 °C. Aldehyde obtained as mention above in 2 mL THF was added via cannula and the solution was warmed up to r.t. overnight (10 h) before quenched with saturated aqueous NH_4Cl solution, extracted with EtOAc (100 mL X 3). The organic layers were combined and dried with anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure vacuum. The residue was purified on a silica gel column using petroleum ether/EtOAc (40/1) as the eluant to afford **18** as a colorless oil (73 mg, 86%). ^1H NMR (400 MHz, $CDCl_3$) δ -0.05 (s, 3 H), 0.02 (s, 3 H), 0.83 (s, 9 H), 1.27 (s, 3 H), 1.43 (s, 3 H), 1.43-1.78 (m, 4 H), 1.92 (b, 1 H), 2.23-2.31 (m, 2 H), 3.76 (m, 1 H), 4.05-4.10 (m, 1 H), 4.43-4.45 (m, 1 H), 5.02-5.05 (m, 2 H), 5.71-5.80 (m, 1 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ -4.30, -4.11, 18.4, 26.0, 26.1, 26.2, 26.5, 26.8, 27.7, 27.8, 28.62, 28.64, 33.2, 33.6, 70.4, 70.5, 71.07, 71.13, 78.6, 78.8, 80.2, 81.78, 81.82, 108.5, 118.6, 118.7,

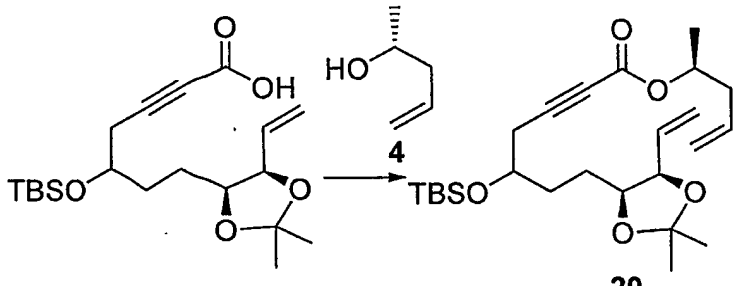
134.7, 134.9. HRMS (FAB) calc'd for $C_{19}H_{34}O_3SiNa^+$ $[M+Na]^+$: 361.2175, found 361.2175, $\Delta = 0.0$ ppm.



18 19

Acid 19:

To a solution of **18** (586 mg, 1.731 mmol) in Et_2O (16 mL) was added BuLi (1.6 M in hexane, 1.817 mmol) at -78 °C and stirred for 5 min before quenched with dry ice and warmed up to r.t. The reaction mixture was washed with NaOH (0.1 M) and the aqueous layers were combined and acidified by HCl (0.1 M) until the pH = 2. The aqueous layer was extracted with EtOAc and the organic layers were combined and dried with anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure vacuum and dried on high vacuum. ^1H NMR (400 MHz, $CDCl_3$) δ -0.03 (s, 3 H), 0.00 (s, 3 H), 0.79 (s, 9 H), 1.17 (s, 3 H), 1.40 (s, 3 H), 0.95-1.68 (m, 4 H), 2.38-2.39 (m, 2 H), 3.80 (b, 1 H), 4.06 (m, 1 H), 4.43 (m, 1 H), 5.14-5.36 (m, 2 H), 5.67-5.76 (m, 1 H), 10.5 (bs, 1 H). This crude was used directly for next step without any further purification.

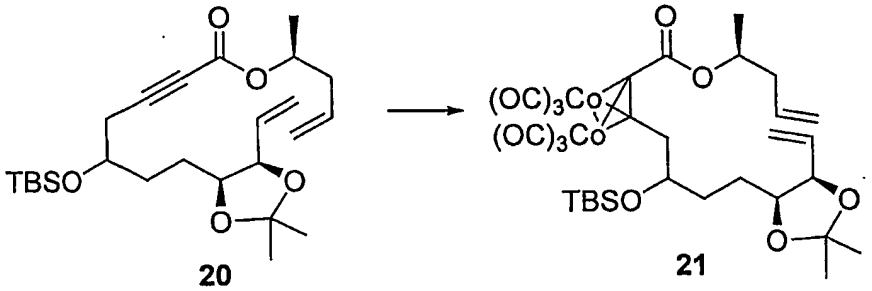


19 20

Ester 20:

To a solution of acid **19** (249 mg, 0.651 mmol) in toluene (15 mL) was added alcohol **4** (0.081 mL, 0.781 mmol), PPh_3 (205 mg, 0.781 mmol), DIAD (0.154 mL, 0.781 mmol).

The reaction mixture was stirred for 10 h and the solvent was removed under reduced pressure vacuum. The residue was purified on a silica gel column using petroleum ether/EtOAc (40/1) as the eluant to afford **20** as a colorless oil (255 mg, 85% for two steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ -0.06-0.00 (m, 6 H), 0.80 (s, 9 H), 1.17 (d, J = 6.2 Hz, 3 H), 1.28 (s, 3 H), 1.38 (s, 3 H), 1.44-1.54 (m, 4 H), 2.20-2.24 (m, 1 H), 2.237-2.31 (m, 1 H), 2.35-2.37 (m, 2 H), 3.78-3.81 (m, 1 H), 4.03-4.05 (m, 1 H), 4.40-4.43 (m, 1 H), 4.94-4.96 (m, 1 H), 5.00-5.04 (m, 2 H), 5.14 (m, 1 H), 5.22 (dd, J = 6.7, 7.1 Hz, 1 H), 5.67-5.72 (m, 2 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ -4.64, -4.58, -4.55, 0.0, 18.0, 19.3, 25.6, 25.7, 25.8, 26.1, 26.3, 27.6, 27.7, 28.2, 28.3, 33.3, 33.6, 40.0, 70.10, 70.12, 72.0, 74.9, 78.1, 78.3, 79.80, 79.82, 86.1, 108.2, 108.3, 118.1, 118.3, 118.4, 133.2, 134.2, 134.4, 153.2. HRMS (FAB) calcd for $C_{25}H_{42}O_5SiH^+$ $[M+H]^+$: 451.2880, found 451.2881, Δ = -0.3 ppm.

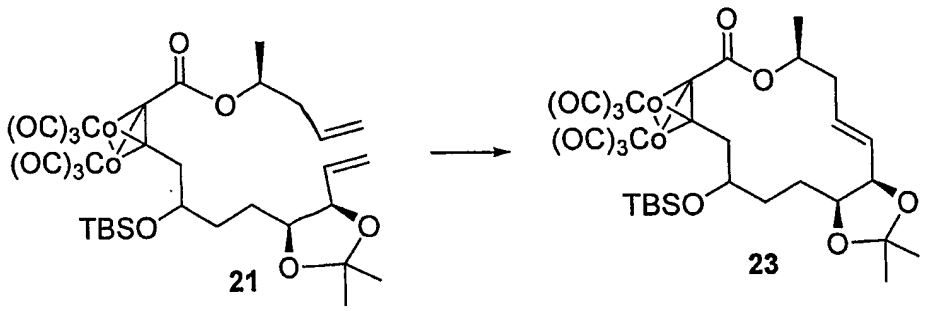


20 **21**

Cobalt-complex 21:

To a solution of **20** (20 mg, 0.044 mmol) in toluene (2.5 mL) was added $Co_2(CO)_8$ (21.2 mg, 0.062 mmol). The reaction mixture was stirred for 30 min before filtered through neutral alumina and concentrated under reduced pressure vacuum. The residue was purified on preparative TLC (Whatman® Pk6F Silica Gel 60 Å 1000 μM) using Hexanes/EtOAc (20/1) as the eluant to afford **21** as a purple oil (30 mg, 94%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.07-0.09 (m, 6 H), 0.90 (s, 9 H), 1.30 (d, J = 8.1 Hz, 3 H), 1.33 (s, 1.6 H), 1.34 (s, 1.4 H), 1.45 (s, 1.6 H), 1.47 (s, 1.4 H), 1.25-1.46 (m, 1 H), 1.58-1.89 (m, 2.5 H), 1.89-1.95 (m, 0.5 H), 2.37-2.40 (m, 2 H), 3.00-3.12 (m, 2 H), 3.82-3.86 (m, 1 H), 4.08-4.14 (m, 1 H), 4.47-4.51 (m, 1 H), 5.07-5.14 (m, 3 H), 5.21 (d, J = 10.4 Hz, 1 H), 5.29 (d, J = 17.1 Hz, 1 H), 5.73-5.84 (m, 2 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 18.1, 19.4, 25.6, 25.8, 26.5, 27.0, 28.16, 28.2, 32.7, 33.0, 40.3, 41.9, 42.1,

71.9, 72.8, 73.0, 78.2, 169.0, 197. HRMS (FAB) calcd for $C_{31}H_{42}Co_2O_{11}SiH^+$ $[M+H]^+$: 737.1239, found 737.1240, Δ = -0.2 ppm.

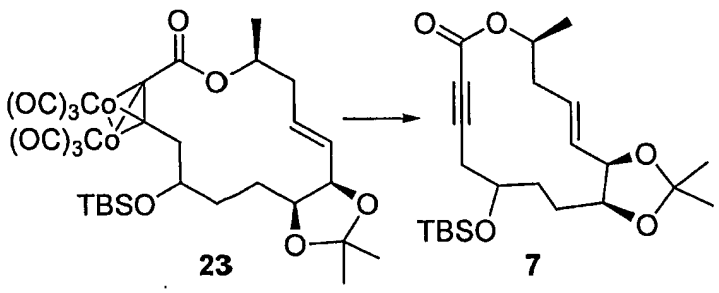


Macrolactone 23:

To a solution of **21** (339 mg, 0.460 mmol) in CH_2Cl_2 (80 mL) was added 2nd generation Grubbs catalyst (97 mg, 0.115 mol) in CH_2Cl_2 (15 mL) via cannula at r.t. The reaction mixture was stirred overnight and then the solvent was removed under reduced pressure vacuum and residue purified on preparative TLC (Whatman® Pk6F Silica Gel 60 Å 1000 μM) using Hexanes/EtOAc (10/1) as the eluant to afford **23A** as a purple oil (123 mg, 38%). $[\alpha]_D^{25}$ -44.7 (*c* 0.25, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) δ 0.08 (s, 3 H), 0.09 (s, 3 H), 0.91 (s, 9 H), 1.31 (d, *J* = 6.3 Hz, 3 H), 1.35 (s, 3 H), 1.45 (s, 3 H), 1.39-1.59 (m, 2 H), 1.65-1.72 (m, 1 H), 1.93-1.97 (m, 1 H), 2.22-2.31 (m, 1 H), 2.42 (dt, *J* = 12.9, 1.83 Hz, 1 H), 3.08 (dd, *J* = 16.0, 1.5 Hz, 1 H), 3.23 (dd, *J* = 16.0, 9.4 Hz, 1 H), 3.92-3.95 (m, 1 H), 4.01-4.06 (m, 1 H), 4.40 (dd, *J* = 9.4, 5.7 Hz, 1 H), 5.53 (ddd, *J* = 15.2, 10.9, 1.52 Hz, 1 H), 5.72 (ddd, *J* = 15.2, 10.7, 3.8 Hz, 1 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ -4.5, -4.4, 18.5, 21.2, 23.1, 26.1, 26.2, 28.8, 30.5, 41.0, 42.7, 71.6, 71.8, 79.3, 80.0, 81.3, 92.9, 108.1, 129.1, 132.6, 169.9, 198.8. HRMS (FAB) calc'd for $C_{29}H_{38}Co_2O_{11}SiH^+$ $[M+H]^+$: 709.0926, found: 709.0924, Δ = 0.2 ppm.

23B (136 mg, 42%). $[\alpha]_D^{25}$ -5.9 (*c* 0.27, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) δ 0.12 (s, 3 H), 0.15 (s, 3 H), 0.93 (s, 9 H), 1.32 (d, *J* = 6.3 Hz, 3 H), 1.35 (s, 3 H), 1.42 (s, 3 H), 1.11-1.54 (m, 2 H), 1.67 (m, 1 H), 1.88-1.93 (m, 1 H), 2.24-2.33 (m, 1 H), 2.41-2.45 (m, 1 H), 3.05 (dd, *J* = 14.9, 9.5 Hz, 1 H), 3.18 (dd, *J* = 14.9, 2.4 Hz, 1 H), 3.54-3.59 (m, 1 H), 4.03-4.09 (m, 1 H), 4.42 (dd, *J* = 9.5 Hz, 6.0 Hz, 1 H), 5.28 (m, 1 H), 5.53 (ddd, *J* = 15.2, 7.9, 4.0 Hz, 1 H), 5.77 (ddd, *J* = 15.2, 10.5, 3.5 Hz, 1 H). ^{13}C

NMR (100 MHz, $CDCl_3$) δ -4.3, -4.0, 18.4, 21.1, 25.8, 26.1, 26.2, 26.3, 26.9, 28.6, 32.4, 40.7, 44.6, 71.8, 73.8, 78.6, 79.8, 81.1, 93.3, 107.9, 128.6, 133.6, 169.6, 198.6. HRMS (FAB) calc'd for $C_{29}H_{38}Co_2O_{11}SiH^+$ $[M+H]^+$: 709.0926, found: 709.0924, Δ = 0.2 ppm.

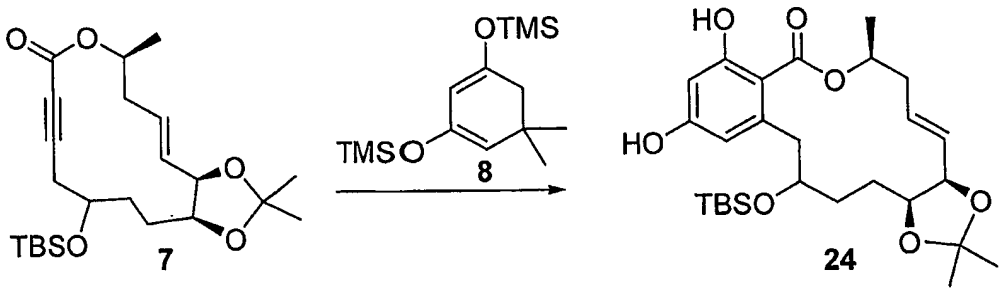


Macrolide 7:

To a solution of **23A** (123 mg, 0.174 mmol) in acetone (10 mL) was added CAN (475 mg, 0.868 mmol) at -10 °C. After 20 min, the reaction mixture was filtered through neutral alumina and the solvent was removed under reduced pressure vacuum. The residue was purified on a silica gel column using petroleum ether/EtOAc (20/1) as the eluant to afford **7A** as a colorless oil (69 mg, 94%). $[\alpha]_D^{25}$ -124.6 (*c* 0.17, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) δ -0.06, (s, 3 H), 0.00 (s, 3 H), 0.81 (s, 9 H), 1.24 (d, J = 6.2 Hz, 1 H), 1.33, (s, 3 H), 1.40 (s, 3 H), 1.59-1.81 (m, 4 H), 2.18-2.22 (m, 1 H), 2.28-2.31 (m, 1 H), 2.34-2.45 (m, 2 H), 3.90-3.93 (m, 1 H), 3.98-4.01 (m, 1 H), 4.33-4.36 (m, 1 H), 4.83-4.87 (m, 1 H), 5.42-5.53 (m, 2 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ -4.8,18.4, 20.6, 26.1, 26.3, 28.4, 28.8, 30.1, 36.5, 40.6, 70.0, 71.7, 78.9, 80.1, 88.5, 108.7, 129.7, 132.0, 153.8.

7B was prepared by same procedure from **23B**. **7B**: (81 mg, 95%). $[\alpha]_D^{25}$ -173.3 (*c* 0.41, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) δ 0.00, (s, 3 H), 0.01 (s, 3 H), 0.81 (s, 9 H), 1.26 (d, J = 6.1 Hz, 3 H), 1.34 (s, 3 H), 1.41 (s, 3 H), 1.53-1.59 (m, 2 H), 1.77-1.81 (m, 2 H), 2.19-2.24 (m, 1 H), 2.24-2.26 (m, 1 H), 2.31-2.47 (m, 2 H), 3.80 (b, 1 H), 3.91-3.93 (m, 1 H), 4.34-4.40 (m, 1 H), 4.75-4.78 (m, 1 H), 4.82-4.86 (m, 1 H), 5.43-5.60 (m, 2 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ -5.0, -4.8, 17.9, 20.17, 20.24, 25.5, 25.6, 25.7, 25.9, 28.0, 28.2, 28.3, 28.4, 29.6, 36.0, 36.7, 40.0, 40.1, 69.5, 70.8, 71.2, 71.4,

78.5, 78.6, 79.4, 79.6, 78.8, 108.2, 129.2, 131.3, 153.0. HRMS (FAB) calc'd for $C_{25}H_{42}O_5SiNa^+$ $[M+Na]^+$: 473.2699, found: 473.2700, Δ = 0.2 ppm.

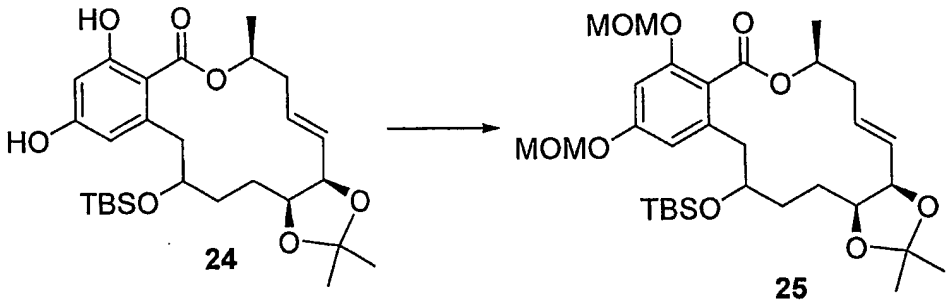


Resorcyclic macrolide 24:

Macrolide **7A** (26 mg, 0.065 mmol) was transferred to a vial and 0.2 mL diene **8** was added. The vial was sealed and heated to 140 0 °C for 36 h. The crude mixture was purified on preparative TLC (Whatman® Pk6F Silica Gel 60 Å 1000 μM) using Hexanes/EtOAc (2/1) as the eluant to afford **24A** as a colorless oil (23 mg, 74%). $[\alpha]_D^{25}$ -99.1 (*c* 0.08, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) δ -0.15, (s, 3 H), 0.09 (s, 3 H), 0.89 (s, 9 H), 1.36 (s, 3 H), 1.38 (s, *J* = 6.1 Hz, 1 H), 1.50 (s, 3 H), 1.25-1.39 (m, 4 H), 1.71-1.76 (m, 1 H), 2.54-2.57 (m, 2 H), 2.60-2.64 (m, 1 H), 3.63-3.68 (m, 2 H), 4.08-4.11 (m, 1 H), 4.48 (m, 1 H), 5.22-5.26 (m, 1 H), 5.54 (bs, 1 H), 5.70-5.75 (m, 2 H), 6.27 (d, *J* = 2.6 Hz, 1 H), 6.28 (d, *J* = 2.6 Hz, 1 H), 11.3 (s, 1 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ -4.5, -4.1, 0.4, 14.6, 18.4, 21.3, 21.5, 24.1, 25.9, 26.3, 28.6, 32.4, 40.1, 42.4, 70.0, 73.5, 73.7, 77.7, 79.7, 101.9, 106.6, 108.7, 111.6, 130.5, 131.6, 145.7, 160.4, 165.0, 172.1. HRMS (FAB) calc'd for $C_{27}H_{42}O_7SiH^+$ $[M+H]^+$: 507.2778, found: 507.2777, Δ = 0.2 ppm.

24B was prepared by same procedure from **7B**. **24B**: (81 mg, 84%). $[\alpha]_D^{25}$ -124.2 (*c* 0.42, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) δ 0.00, (s, 3 H), 0.14 (s, 3 H), 0.99 (s, 9 H), 1.50 (s, 3 H), 1.58 (d, *J* = 6.1 Hz, 1 H), 1.40-1.67 (m, 2 H), 1.64 (s, 3 H), 1.87-1.92 (m, 2 H), 2.64-2.72 (m, 2 H), 3.09 (dd, *J* = 3.8 Hz, 2.5 Hz, 1 H), 3.45 (dd, *J* = 13.8, 7.8 Hz, 1 H), 4.02-4.04 (m, 1 H), 4.20-4.22 (m, 1 H), 4.70-4.74 (m, 1 H), 5.50-5.52 (m, 1 H), 5.76 (dd, *J* = 15.5, 8.1 Hz, 1 H), 5.93-5.95 (m, 2 H), 6.42 (m, 1 H), 6.48 (m, 1 H), 11.62

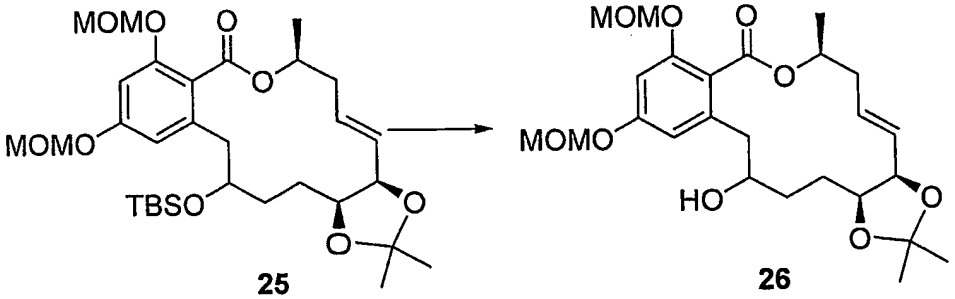
(s, 1 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ -4.3, -4.0, 14.5, 18.3, 18.4, 20.5, 21.5, 25.8, 26.2, 28.3, 28.4, 28.5, 33.1, 61.1, 72.7, 73.5, 79.1, 102.1, 107.1, 108.5, 112.7, 130.1, 130.5, 144.2, 160.7, 164.9, 171.7. LRMS (ESI) calcd for $C_{27}H_{42}O_7SiNa^+$ $[M+Na]^+$: 529.2, found: 529.1. LRMS (ESI) calcd for $C_{27}H_{42}O_7SiCl^-$ $[M+Cl]^-$: 541.3, found: 541.2.



MOM ether 25:

To a solution of **24A** (23 mg, 0.045 mmol) in CH_2Cl_2 (0.5 mL) was added diethylpropylethylamine (0.08 mL, 0.450 mmol) and MOMCl (0.018 mL, 0.227 mmol). The reaction mixture was stirred for 10 h before quenched with saturated aqueous NH_4Cl solution, extracted with EtOAC (100 mL X 3). The organic layers were combined and dried with anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure vacuum. The residue was purified on preparative TLC (Whatman® Pk6F Silica Gel 60 Å 1000 μM) using Hexanes/EtOAc (2/1) as the eluant to afford **25A** as a colorless oil (21 mg, 78%). $[\alpha]_D^{25}$ -2.86 (*c* 0.07, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) δ 0.00, (s, 3 H), 0.10 (s, 3 H), 0.93 (s, 9 H), 1.39 (s, 3 H), 1.39 (d, 3 H), 1.48 (s, 3 H), 1.42-1.48 (m, 1 H), 1.60-1.64 (m, 2 H), 1.72-1.77 (m, 1 H), 2.41-2.45 (m, 2 H), 2.66 (dd, J = 5.6, 4.6 Hz, 1 H), 2.89 (dd, J = 4.5, 1.6 Hz, 1 H), 3.47 (s, 3 H), 3.48 (s, 3 H), 3.97-3.98 (m, 1 H), 4.09-4.15 (m, 1 H), 4.73 (dd, J = 9.0, 6.0 Hz, 1 H), 5.13-5.19 (m, 4 H), 5.30-5.36 (m, 1 H), 5.57 (dd, J = 15.4, 9.1 Hz, 1 H), 5.70-5.77 (m, 1 H), 6.68 (d, J = 2.0 Hz, 1 H), 6.86 (d, J = 2.0 Hz, 1 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ -4.3, -4.2, 0.5, 18.4, 21.6, 24.0, 25.9, 26.3, 28.7, 30.1, 32.3, 40.1, 40.4, 56.5, 56.6, 71.4, 71.6, 80.3, 94.7, 94.9, 101.8, 108.5, 111.4, 119.8, 130.2, 132.4, 139.4, 155.3, 158.6, 168.4.

25B was prepared by same procedure from **24B**. **25B**: (58 mg, 83%). $[\alpha]_D^{25}$ -16.2 (*c* 0.29, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) δ -0.17 (s, 3 H), -0.06 (s, 3 H), 0.81 (s, 9 H), 1.26 (s, 3 H), 1.33 (d, *J* = 6.1 Hz, 1 H), 1.38 (s, 3 H), 1.17-1.52 (m, 4 H), 2.30-2.36 (m, 2 H), 2.61 (dd, *J* = 14.2, 6.0 Hz, 1 H), 2.71 (dd, *J* = 14.2, 6.9 Hz, 1 H), 3.39 (s, 3 H), 3.40 (s, 3 H), 3.80-3.83 (m, 1 H), 4.10-4.14 (m, 1 H), 4.41 (dd, *J* = 8.9, 6.1 Hz, 1 H), 5.04-5.09 (m, 4 H), 5.20-5.23 (m, 1 H), 5.43 (dd, *J* = 15.3, 9.1 Hz, 1 H), 5.60-5.67 (m, 1 H), 6.46 (s, 1 H), 6.60 (s, 1 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ -4.2, -3.8, 18.4, 21.3, 25.8, 26.2, 26.25, 26.3, 28.6, 33.0, 39.8, 40.0, 42.0, 56.5, 56.6, 71.7, 74.5, 78.6, 79.9, 94.7, 94.9, 101.5, 108.3, 111.2, 119.8, 131.0, 131.4, 139.2, 155.8, 158.9, 168.5. HRMS (FAB) calc'd for $C_{31}H_{50}O_9SiH^+$ $[M+H]^+$: 595.3302, found: 595.3304, Δ = -0.3 ppm.



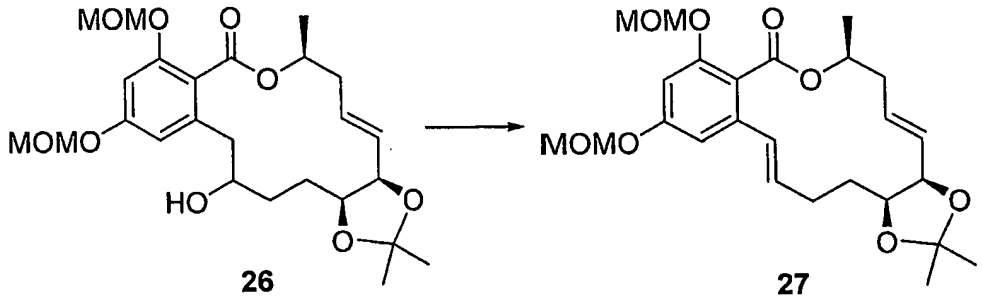
25 **26**

Alcohol 26:

To a solution of **25A** (21 mg, 0.035 mmol) in THF (1.4 mL) was added pyridine (0.6 mL) and HF-pyridine (30%, 0.3 mL). The reaction mixture was stirred for 10 h before quenched with MeOTf (2 mL) and stirred for 1 h. The solvent was removed under reduced pressure vacuum. The residue was purified on preparative TLC (Whatman® Pk6F Silica Gel 60 Å 1000 μM) using Hexanes/EtOAc (1/1) as the eluant to afford **26A** as a colorless oil (12 mg, 78%). $[\alpha]_D^{25}$ -105.2 (*c* 0.06, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) δ -0.05 (s, 3 H), 0.05 (s, 3 H), 1.35 (s, 3 H), 1.38 (d, *J* = 6.2 Hz, 3 H), 1.46 (s, 3 H), 1.61-1.81 (m, 4 H), 2.40-2.46 (m, 2 H), 2.70 (dd, *J* = 14.1, 6.1 Hz, 1 H), 2.81 (dd, *J* = 14.1, 4.7 Hz, 1 H), 3.46 (s, 6 H), 3.89 (b, 1 H), 4.10-4.14 (m, 1 H), 4.56 (dd, *J* = 9.1, 6.1 Hz, 1 H), 5.13-5.17 (m, 4 H), 5.32-5.37 (m, 1 H), 5.59 (dd, *J* = 15.4, 9.2 Hz, 1 H), 5.70-5.75 (m, 1 H), 6.66 (d, *J* = 2.0 Hz, 1 H), 6.70 (d, *J* = 2.0 Hz, 1 H). ^{13}C NMR (100

MHz, $CDCl_3$) δ 21.5, 25.1, 25.7, 28.6, 30.1, 32.3, 40.0, 41.6, 56.6, 56.7, 70.5, 72.0, 80.1, 94.7, 94.9, 101.9, 108.2, 111.0, 119.7, 130.6, 132.7, 138.3, 155.8, 159.1, 168.4. HRMS (FAB) calc'd for $C_{25}H_{36}O_9H^+$ $[M+H]^+$: 482.2438, found: 482.2437, Δ = 0.1 ppm.

26B was prepared by same procedure from **25B**. **26B**: (20 mg, 87%). $[\alpha]_D^{25}$ -32.0 (*c* 0.10, $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$) δ 1.18 (m, 2 H), 1.35 (s, 3 H), 1.37 (d, *J* = 6.2 Hz, 1 H), 1.42 (s, 3 H), 1.55-1.69 (m, 2 H), 1.98-2.06 (m, 1 H), 2.42-2.48 (m, 3 H), 2.78 (dd, *J* = 13.8, 2.4 Hz, 1 H), 3.41-3.50 (m, 6 H), 3.62-3.67 (m, 1 H), 4.19-4.25 (m, 1 H), 4.49 (dd, *J* = 9.4, 6.0 Hz, 1 H), 5.11-5.18 (m, 4 H), 5.36-5.42 (m, 1 H), 5.56 (dd, *J* = 15.4, 9.5 Hz, 1 H), 5.65-5.72 (m, 1 H), 6.57 (d, *J* = 2.0 Hz, 1 H), 6.66 (d, *J* = 2.0 Hz, 1 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 21.6, 25.8, 26.8, 28.6, 32.0, 39.8, 42.6, 56.5, 56.7, 72.3, 74.8, 80.3, 94.7, 94.9, 101.8, 108.6, 110.7, 108.6, 110.7, 119.5, 129.9, 132.8, 138.7, 155.9, 159.0, 168.7. HRMS (FAB) calc'd for $C_{25}H_{36}O_9H^+$ $[M+H]^+$: 482.2438, found: 482.2437, Δ = 0.1 ppm.

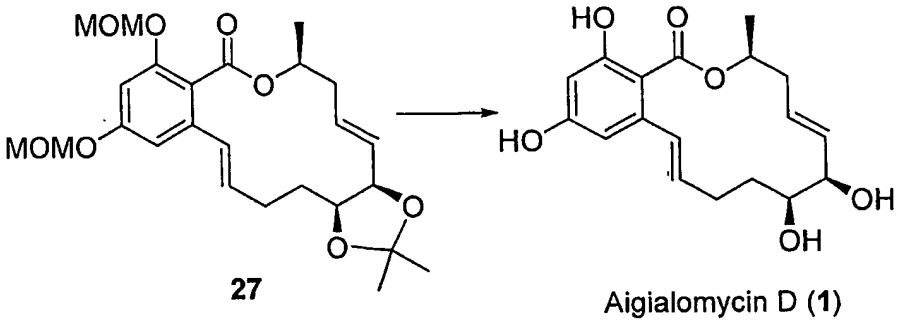


Diene 27:

A solution of Martin's sulfurane dehydration agent (140 mg, 0.208) was added into a vial containing **26B** (20 mg, 0.042) at 0 °C. The reaction mixture was warmed up to r.t. over 2 h and the crude was purified on preparative TLC (Whatman® Pk6F Silica Gel 60 Å 1000 μM) using Hexanes/EtOAc (1/1) as the eluant to afford **27** as a colorless oil (16 mg, 84%). $[\alpha]_D^{25}$ -123.8 (*c* 0.08, $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$) δ 1.35 (s, 3 H), 1.36 (d, *J* = 6.0 Hz, 3 H), 1.46 (s, 3 H), 1.49-1.55 (m, 1 H), 1.80-1.85 (m, 1 H), 2.07-2.11 (m, 1 H), 2.29-2.32 (m, 1 H), 2.45-2.55 (m, 2 H), 3.41-3.50 (m, 6 H), 4.16-4.21 (m, 1 H), 4.56 (dd, *J* = 9.5, 5.4 Hz, 1 H, 1 H), 5.10-5.20 (m, 4 H), 5.32-5.36 (m, 1 H),

5.59 (dd, J=15.5, 9.6 Hz, 1 H), 5.70-5.77 (m, 1 H), 5.15 (m, 1 H), 6.24 (d, J= 15.4 Hz, 1 H), 6.80 (d, J = 1.8 HZ, 1 H), 6.68 (d, J = 1.8 HZ, 1 H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 21.1, 25.8, 28.6, 28.7, 29.0, 39.5, 71.6, 80.1, 84.3, 94.6, 102.6, 104.8, 108.3, 117.9, 124.8, 128.4, 129.3, 131.9, 132.3, 136.8, 155.1, 158.9, 167.3. HRMS (FAB) calcd for $C_{25}H_{34}O_8H^+$ $[M+H]^+$: 463.2332, found: 463.2333, Δ = -0.2 ppm.

27 could also be obtained from **26A** using same procedure (90%).



27 Aigialomycin D (**1**)

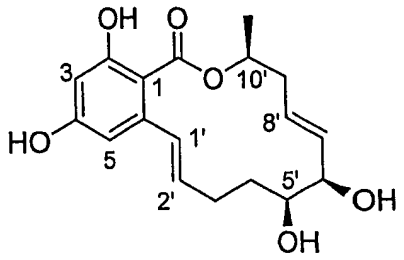
Aigialomycin D (1):

To a solution of **27** (16 mg, 0.035) in MeOH (1.5 mL) was added HCl (1 *N*, 1.5 mL) and stirred for 2 d. The reaction was quenched with saturated aqueous $NaHCO_3$ solution and extracted with EtOAc. The organic layers were combined and dried over anhydrous $MgSO_4$, filtered and concentrated under reduced vacuum. The crude was purified on preparative TLC (Whatman® Pk6F Silica Gel 60 Å 1000 μM) using $MeOH/CH_2Cl_2$ (5%) as the eluant to afford **1** as a white solid (8 mg, 69%). Mp: 84.2-86.9 °C. $[\alpha]_D^{25}$ -18.0 (*c* 0.03, MeOH). IR (neat) 3346, 1643, 1607, 1311, 1261, 1166, 1017, 968. ^{1}H NMR (500 MHz, acetone-d_6) δ 1.39 (d, J= 6.4 Hz, 3 H), 1.58-1.61 (m, 1 H), 2.14 (m, 1 H), 2.32-2.36 (m, 2 H), 2.43-2.46 (m, 1 H), 2.57 (ddd, J= 14.5, 7.3, 3.1 Hz, 1 H), 3.56 (br, 1 H), 3.64 (m, 1 H), 3.76 (br, 1 H), 4.35 (brd, J= 4.1 Hz, 1 H), 5.41-5.47 (m, 1 H), 5.69 (dd, J= 15.6, 5.1 Hz, 1 H), 5.87 (dddd, J= 15.6, 7.4, 7.4, 1.4 Hz, 1 H), 6.10 (ddd, J= 15.9, 5.5, 5.7 Hz, 1 H), 6.28 (d, J= 2.3 Hz, 1 H), 6.53 (d, J= 2.3 Hz, 1 H), 7.16 (d, J= 15.9 Hz, 1 H), 9.10 (bs, 1 H), 11.7 (s, 1 H). ^{13}C NMR (125 MHz, acetone-d_6) δ 19.2, 28.1, 28.8, 38.1, 73.1, 73.4, 76.7, 102.6, 104.6, 107.9, 125.6, 130.8, 133.8, 135.9, 144.5, 163.2, 166.0, 172.3. LRMS (ESI) calcd for $C_{18}H_{22}O_6Na^+$ $[Na+H]^+$: 357.1, found: 357.3. LRMS (ESI) calcd for $C_{18}H_{21}O_6^-$ $[M-H]^-$: 333.1, found:

333.1. LRMS (ESI) calcd for $C_{18}H_{22}O_6Cl^-$ $[M+Cl]^-$: 369.1, found: 369.0. HRMS (TOF) calcd for $C_{18}H_{22}O_6Na^+$ $[M+Na]^+$: 357.1314, found: 357.1325, $\Delta = 3.1$ ppm. All the physical data are consistent with the reported value (Isaka, M.; Suyarnsestakorn, C.; Tanticharoen, M.; Kongsaeree, P.; Thebtaranonth, Y. *J. Org. Chem.* **2002**, *67*, 1561-1566; incorporated herein by reference).

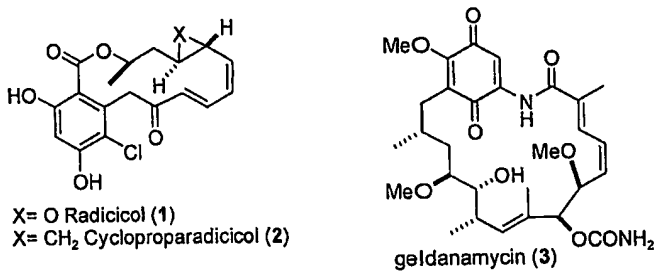
^{1}H NMR Data of Aigialomycin D

position	Isolated Aigialomycin D by Isaka	Synthetic Aigailomycin D
3	6.27 (d, 2.4)	6.28 (d, 2.3)
5	6.52 (d, 2.4)	6.53 (d, 2.3)
1'	7.14 (d, 15.9)	7.16 (d, 15.9)
2'	6.09 (ddd, 15.9, 5.6, 5.4)	6.10 (ddd, 15.9, 5.7, 5.5)
3'	2.31-2.34 (m)	2.32-2.36 (m)
	2.31-2.34 (m)	2.32-2.36 (m)
4'	2.14 (m)	2.14 (m)
	1.58 (m)	1.58-1.61 (m)
5'	3.62 (m)	3.64 (m)
6'	4.35 (brd, 4.3)	4.35 (brd, 4.1)
7'	5.68 (dd, 15.7, 5.0)	5.69 (dd, 15.6, 5.1)
8'	5.87 (dddd, 15.7, 7.3, 7.3, 1.2)	5.87 (dddd, 15.6, 7.4, 7.4, 1.4)
9'	2.55 (ddd, 14.6, 7.5, 3.2)	2.55 (ddd, 14.5, 7.3, 3.1)
	2.42 (m)	2.43-2.46 (m)
10'	5.42 (m)	5.41-5.47 (m)
10'-C*H*$_3$	1.38 (d, 6.4)	1.39 (d, 6.4)
2-O*H*	11.65 (s)	11.7 (s)
4-O*H*	9.5 (br)	9.1 (br)
5'-O*H*	not detected	3.56 (br)
6'-O*H*	not detected	3.76 (br)



aigialomycin D

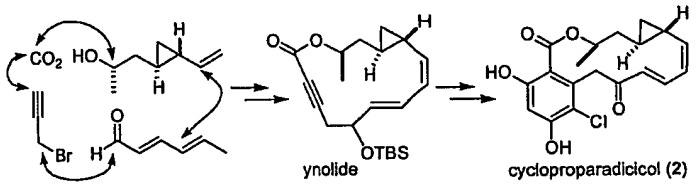
Example 2-Concise Route to Benzofused Macrolactones via Ynolides: Cycloproparadicicol



Structures of Hsp90 Inhibitors

In this Example, we report a new approach to the broad family of resorcinylic fused macrolides. The underlying concept is captured graphically in Scheme 2-1, which is directed to our focusing target, cycloproapradicicol (**2**). However, as is suggested by the very facile synthesis of model compound **13** (*vide infra*), and has been further established in ongoing work, the method is quite general. The central element of our plan is the building of an "ynolide" intermediate and its advancement to the benzomacrolide by a Diels-Alder cycloaddition. The ynolide is constructed through olefin metathesis, enabled only by presentation of the acetylene linakge as its dicobalt hexacarbonyl cluster (see **9** → **10** and **14** → **15**) (Young, D. G.; Burlison, J. A.; Peters, U. *J. Org. Chem.* **2003**, *68*, 3494; incorporated herein by reference).

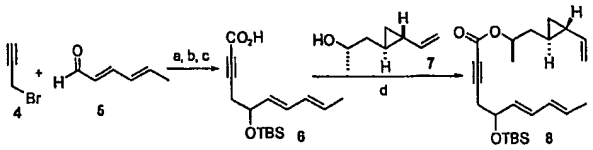
Scheme 2-1. New Synthetic Strategy



Our synthesis commenced with commercial 2,4-hexadienal (sorbaldehyde, **5**, Scheme 2-2). Reformatsky-like condensation of propargyl bromide (**4**) with **5**, followed by TBS ether protection and subsequent reaction of the lithium alkynide ion with CO_2, provided acid **6**. Following reaction of racemic **6** and the known optically pure and defined alcohol **7** (Yamamoto, K.; Gabaccio, R. M.; Stachel, S. J.; Solit, D.

B.; Chiosis, G., Rosen, N.; Danishefsky, S. J. *Angew. Chem. Int. Ed.* **2003**, *42*, 1280; incorporated herein by reference) under Mitsonobu conditions, ester **8** was obtained.

Scheme 2-2. Synthesis of the Acyclic Alkynoic Ester

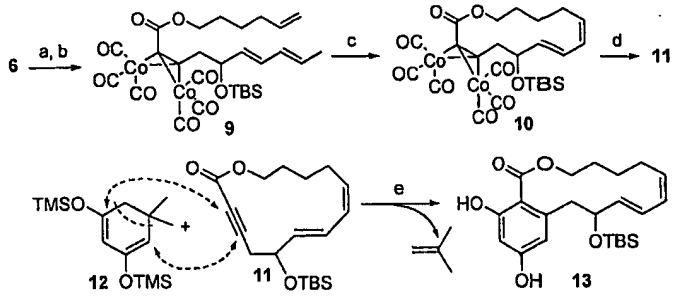


Reagents and conditions: (a) (i) Zn, THF, 66%; (b) TBSCl, imidazole, DMAP, CH_2Cl_2, 100%; (c) BuLi, -78 °C; then CO_2; (d) DIAD, Ph_3P, THF, -20 °C, 47% (two steps).

Projected ring-closing metathesis (RCM) reactions were conducted with a cyclic alkyne. Unfortunately, triene **8** failed to cyclize under a variety of RCM conditions. We took this negative finding to reflect impediments to cyclization arising from the linear character of the acetylene, possibly aggravated by rigidities associated with the trans-disubstituted cyclopropane. A more flexible model compound was prepared from acid **6** and 5-hexen-1-ol, and subjected to RCM reactions (Scheme 2-3). Again, only starting material was recovered. Aside from the constraint to cyclization imposed by linear alkyne, the cyclization could further be complicated by non productive coordination of the acetylene to the RCM catalytic machinery. It is well known that reaction of dicobalt carbonyl with acetylenes can lead to stable complexes (Greenfield, H.; Sternberg, H, W,; Friedel, R. A.; Wotiz, J. H.; Markby, R.; Wender, I. *J. Am. Chem. Soc.* 1956, 78, 120; Nicholas, K. M.; Pettit, R. *Tetrahedron Lett.* **1971**, 3475; each of which is incorporated herein by reference), wherein the geometry of cobalt-complexed alkynes is distorted to approximately 140° (Dickson, R. S.; Fraser, P. J. *Adv. Organomet. Chem.* **1974**, *12*, 323; incorporated herein by reference).

In the event, cyclization of **9** proceeded smoothly under the conditions shown. Following oxidative removal of the cobalt using ammonium cerium (IV) nitrate (CAN), the desired cyclic alkynoic ester **11** was generated in high yield (Scheme 2-3).

Scheme 2-3. Synthesis of the Model Resorcinylic Macrolactone

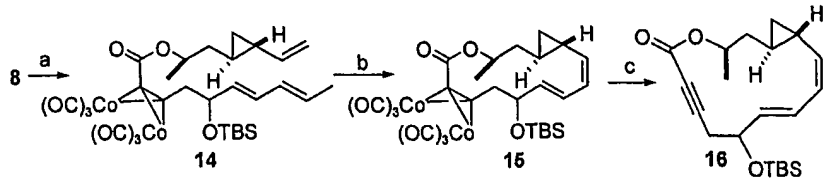


Reagents and conditions: (a) 5-hexen-1-ol, EDC/DMAP, CH_2Cl_2, 59%; (b) $Co_2(CO)_8$, PhMe, 86%; (c) 2nd generation Grubbs catalyst (25 mol%), CH_2Cl_2 (0.2 mM), 45 °C, 71%; (d) CAN, acetone, -10 °C, 92%; (e) 140 °C, neat; then SiO_2, 60%.

Construction of the resorcinylic skeleton called for a Diels-Alder reaction of **11** with a 1,3-bis-oxygenated diene. We found that the known dimedone-derived diene, 5,5-dimethyl-1,3-bis-trimethylsilyloxy-cyclohexa-1,3-diene (Ibuka, T.; Mori, Y.; Aoyama, T.; Inubushi, Y. *Chem. Pharm. Bull.* **1978**, *26*, 456; Langer, P.; Schneider, T.; Stoll, M. *Chem. Eur. J.* **2000**, *6*, 320; each of which is incorporated herein by reference) (**12**, Scheme 2-3), served our purpose best. Indeed, Diels-Alder reaction of cyclic alkyne **11** with **12** proceeded smoothly at 140 °C, providing the desired aromatic product **13** in 60% yield, after concomitant retro-Diels-Alder loss of isobutene from the initial adduct, and hydrolysis of the trimethylsilyl ether groups during chromatography.

We applied this strategy to the targeted system (**8**). Gratifyingly, under the same RCM conditions, cyclopropane-containing cobalt complex **14** cyclized to give **15** in 57% yield, as a 2:1 mixture of two diatereomers (Scheme 2-4) (Here, we described only the conversion of the major isomer of **15** to **2**. The other isomer worked equally well). In this case, removal of cobalt on **15**, however, proved to be challenging, presumably due to the presence of the sensitive vinyl cyclopropane functionality. After screening a variety of conditions, we found that I_2-THF worked well (Tanaka, S.; Tsukiyama, T.; Isobe, M. *Tetrahedron Lett.* **1993**, *34*, 5757; incorporated herein by reference). The key cyclic alkyne dienophile **16** was thus obtained in 69% yield.

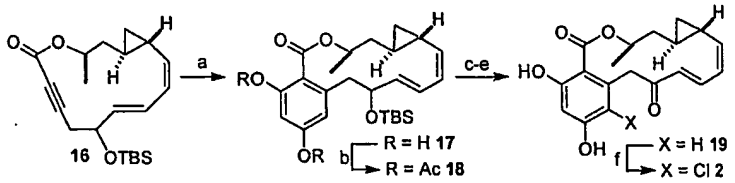
Scheme 2-4. Synthesis of the Ynolide



Reagents and conditions: (a) $Co_2(CO)_8$, PhMe, 100%; (b) 2nd genera-tion Grubbs catalyst (25 mol%), CH_2Cl_2 (0.2 mM), 45 °C, 57%; (c) I_2, THF, 0 °C, 69%.

Diels-Alder reaction of **16** with diene **12** furnished the desired product **17** in 75% yield (Scheme 2-5). Transformation of **17** to the desired ketone by direct oxidation turned out to be a non-trival matter. In the end, it was accomplished following protection of the two phenolic functions, as shown, by straightford transformations to afford dechlorinated analogue **19** (Scheme 2-5). Finally, regioselective chlorination of **19** using SO_2Cl_2 in CH_2Cl_2 (Yamamoto, K.; Gabaccio, R. M.; Stachel, S. J.; Solit, D. B.; Chiosis, G., Rosen, N.; Danishefsky, S. J. *Angew. Chem. Int. Ed.* **2003**, *42*, 1280; Garbaccio, R. M.; Stachel, S. J.; Baseschlin, D. K.; Danishefsky, S. J. *J.. Chem. Soc.* **2001**, *123*, 1090; each of which is incorporated herein by reference), converted **19** into cycloproparadicicol (**2**).

Scheme 2-5. Completion of the Synthesis

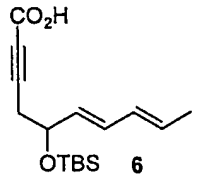


Reagents and conditions: (a) **12**, 140 °C, neat, 75%; (b) Ac_2O, DMAP, DMF, 87%; (c) HF/Pyr. THF; (d) Dess-Martin periodinane, CH_2Cl_2, 68% (two steps); (e) 5% $NaHCO_3$/MeOH, 92%; (f) SO_2Cl_2, CH_2Cl_2, 0 °C, 61%.

In summary, a new efficient synthetic route has been developed for a pre-clinical candidate, cycloproparadicicol (**2**) and, by extension, to a broad range of benzofused macrolactones.

Experimentals:

General Methods: Reagents obtained from commercial suppliers were used without further purification unless otherwise noted. THF, toluene, and methylene chloride was obtained from a dry solvent system (passed through a prepacked column of alumina) and used without further drying. All air and water sensitive reactions were performed in oven or flame-dried glassware. NMR (^1H and ^{13}C) spectra were recorded on Bruker AMX-400 MHz or Bruker Advance DRX-500 MHz as noted individually, referenced to $CDCl_3$ (7.27 ppm for ^1H and 77.23 ppm for ^{13}C). Optical rotations were obtained on a JASCO model DIP-370 digital polarimeter. Low resolution mass spectra (ESI) were determined with a PESciex AP 130 spectrometer. High resolution mass spectra (FAB) were determined at Chemistry Department of Columbia University. Flash chromatography was performed with silica gel (230-400 mesh) from EM Science as the stationary phase. Analytical thin-layer chromatography was performed on E. Merck silica gel 60 F254 plates. Compounds which were not UV active were visualized by dipping the plates in phosphomolybdic acid solution and heating. Preparative thin layer chromatography was performed using the indicated solvent on Whatman® (LK6F Silica gel 60 Å 250 μM or Pk6F Silica Gel 60 Å 1000 μM) TLC plate.

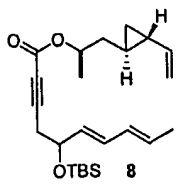


6

Acid **6**. To a suspension of activated zinc (15 g, 230 mmol) in dry THF (50 mL) at 0 °C was added propargyl bromide **4** (19.2 mL 80 wt% in toluene, 172 mmol). The resulting mixture was stirred at 0 °C for 1 hr, and sorbaldehyde **5** (12.7 mL, 115 mmol) was added. After 1 hr at 0 °C, additional zinc (4.5 g, 69 mmol) was added, and stirring was continued for 2.5 hrs at room temperature (the reaction was exothermic and ice bath was needed occasionally to keep the temperature down). The reaction was quenched by slow addition of sat. aqueous NH_4Cl (500 mL), followed by diluting with Et_2O (1 L). The layers were separated, and the organic layer was washed with H_2O

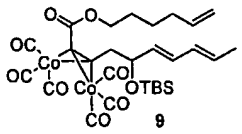
(300 mL), brine (300 mL), dried (Na_2SO_4), filtered and concentrated in vacuum. The residue was dissolved in CH_2Cl_2 (750 mL) with imidazole (9.8 g, 144 mmol), *t*-butyldimethylsilyl chloride (19 g, 126 mmol) and 4-(dimethylamino) pyridine (1.4 g, 11.5 mmol), and stirred at room temperature for 3 hrs. Additional imidazole (4.9 g, 72 mmol) and *t*-butyldimethylsilyl chloride (9.5 g, 63 mmol) were added, and stirring was continued for 9 hrs. The reaction was quenched by addition of sat. aqueous NH_4Cl (200 mL). The layers were separated, and the organic layer was washed with H_2O (200 mL), brine (200 mL), dried (Na_2SO_4), filtered and concentrated in vacuum. The residue was purified by flash chromatography (silica, 0 to 10% Et_2O in hexane) to give the terminal alkyne precursor of **6** (15 g, 52%). ^1H NMR ($CDCl_3$, 400 MHz) δ 6.18 (dd, J = 15.1, 10.5 Hz, 1H), 6.03 (ddd, J = 15.0, 10.6, 1.3 Hz, 1H), 5.72 (dd, J = 14.9, 6.9 Hz, 1H), 5.61 (dd, J = 15.1, 6.4 Hz, 1H), 4.30 (q, J = 6.3 Hz, 1H), 2.43 (ddd, J = 16.5, 6.2, 2.7 Hz, 1H), 2.34 (ddd, J = 16.5, 6.8, 1.7 Hz, 1H), 2.60 (t, J = 2.6 Hz, 1H), 1.77 (d, J = 6.8 Hz, 3H), 0.91 (s, 9H), 0.09, 0.06 (2s, 6H); ^{13}C NMR ($CDCl_3$, 100 Hz) δ 132.5, 131.1, 130.7, 130.0, 81.7, 72.1, 70.1, 28.9, 26.1, 18.4, -2.7; IR (film) ν_{max} 3313, 2956, 2930, 2856, 2121, 1255, 1115, 1079, 987, 837; ESIMS *m/z* 273 ([M + Na^+], $C_{15}H_{26}NaOSi$ requires 273).

To a solution of the terminal alkyne precursor of **6** (15.0 g, 59.9 mmol) in Et_2O (270 mL) at -78 °C, was added a solution of BuLi (1.6 M in hexane, 41.5 mL, 66.4 mmol). After 45 min, excess crushed dry ice was added and the reaction was allowed to warm to room temperature. The solution was acidified by addition of 0.5 M aqueous citric acid (300 mL). The layers were separated, and the aqueous layer was extracted with additional Et_2O (300 mL x 2). The combined organic layers were dried ($MgSO_4$), filtered and concentrated in vacuum. The residue was purified by flash chromatography (silica, 50% to 100% EtOAc in hexane) to give the product as a light yellow solid (17.5 g, 99%). ^1H NMR ($CDCl_3$, 400 MHz) δ 6.19 (dd, J = 15.1, 10.4 Hz, 1H), 6.03 (ddd, J = 14.9, 10.6, 1.3 Hz, 1H), 5.75 (dd, J = 15.0, 6.8 Hz, 1H), 5.55 (dd, J = 15.1, 6.4 Hz, 1H), 4.36 (q, J = 6.3 Hz, 1H), 2.61-2.48 (m, 2H), 1.77 (d, J = 6.8 Hz, 3H), 0.91 (s, 9H), 0.10, 0.06 (2s, 6H); ^{13}C NMR ($CDCl_3$, 100 Hz) δ 157.6, 131.6, 131.3, 130.7, 89.7, 74.0, 71.4, 29.3, 26.0, 18.4, 18.3, -4.3, -4.7; IR (film) ν_{max} 2956, 2930, 2857, 2242, 1689, 1281, 1257, 1080; ESIMS *m/z* 317 ([M + Na^+], $C_{16}H_{26}NaO_3Si$ requires 317).



8

Alkynoic ester **8**. To a solution of DIAD (14.7 mL, 72.9 mmol) in dry THF (350 mL) was added Ph_3P (15.8 g, 60.2 mmol), and the mixture was stirred at room temperature for one hour. At -20 °C, a solution of acid **6** (13.1 g, 44.4 mmol) in 100 mL THF was added. After 15 min, a solution of alcohol **7** (4.0 g, 31.7 mmol) in 150 mL THF was added, and stirring was continued for 2 hours at -20 °C. The reaction was quenched by addition of 250 mL of pH 7.2 phosphate buffer, followed by warming to room temperature and diluting with EtOAc (1.5 L). The layers were separated, and the aqueous layer was extracted with EtOAc (2 x 250 mL). The combined organic layers were washed with brine (250 mL), dried (Na_2SO_4), filtered and concentrated in vacuum. The residue was purified by flash chromatography (silica, 50:1→20:1 hexanes/EtOAc) to give ester **8** as a mixture of two inseparable diastereoisomers (5.9 g, 47%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.17 (dd, J = 15.1, 10.5 Hz, 1H), 6.03 (ddd, J = 12.3, 10.6, 1.4 Hz, 1H), 5.70 (dd, J = 14.8, 6.8 Hz, 1H), 5.55 (dd, J = 15.2, 6.4 Hz, 1H), 5.37 (ddd, J = 17.1, 10.2, 8.7 Hz, 1H), 5.06 (q, J = 6.4 Hz, 1H), 5.03 (dd, J = 17.0, 1.5 Hz, 1H), 4.84 (dd, J = 10.2, 1.6 Hz, 1H), 4.34 (q, J = 6.4 Hz, 1H), 2.56-2.42 (m, 2H), 1.76 (d, J = 6.8 Hz, 3H), 1.57-1.53 (m, 2H), 1.29 (d, J = 6.4 Hz, 3H), 1.20-1.10 (m, 1H), 0.90 (s, 9H), 0.79-0.68 (m, 1H), 0.65-0.57 (m, 2H), 0.10, 0.05 (2s, 6H); ^{13}C NMR ($CDCl_3$, 100 Hz) δ 154.5, 141.4, 131.8, 131.1, 130.8, 130.4, 112.1, 86.2, 74.9, 73.0, 71.5, 39.7, 29.2, 26.0, 22.4, 19.8, 18.4, 18.3, 17.2, 13.7, -4.3, -4.7; IR (film) ν_{max} 2955, 2930, 2856, 2238, 1710, 1253, 1068; ESIMS *m/z* 437 ([M + Cl$^-$], $C_{24}H_{38}ClO_3Si$ requires 437); HRMS (FAB$^+$) *m/z* 403.2687 ([M + H]$^+$, $C_{24}H_{39}O_3Si$ requires 403.2668).

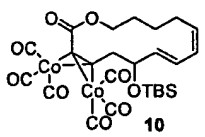


9

Cobalt complex **9**. To a solution of acid **6** (192 mg, 0.653 mmol) and 5-hexen-1-ol (0.118 mL, 0.979 mmol) in dry CH_2Cl_2 (3 mL) was added EDCI (150 mg, 0.784 mmol)

and 4-(dimethylamino)pyridine (8.0 mg, 0.065 mmol). After 3 hrs at room temperature, the reaction mixture was loaded on PTLC plates and purified (12:1 hexane/EtOAc) to give the model ester (146 mg, 59%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.18 (dd, J = 15.1, 10.5 Hz, 1H), 6.04 (ddd, J = 15.0, 10.5, 1.5 Hz, 1H), 5.79 (ddt, J = 17.1, 10.3, 7.2 Hz, 1H), 5.71 (dq, J = 15.0, 6.8 Hz, 1H), 5.56 (dd, J = 15.1, 6.4 Hz, 1H), 5.03 (dq, J = 17.1, 1.6 Hz, 1H), 4.97 (dd, J = 10.2, 1.6 Hz, 1H), 4.35 (q, J = 6.3 Hz, 1H), 4.16 (t, J = 6.6 Hz, 2H), 2.57-2.44 (m, 2H), 2.09 (q, J = 7.2 Hz, 1H), 1.77 (d, J = 6.9 Hz, 3H), 1.74-1.63 (m, 2H), 1.52-1.44 (m, 2H), 0.90 (s, 9H), 0.10, 0.06 (2s, 6H); ^{13}C NMR ($CDCl_3$, 100 Hz) δ 154.0, 138.4, 131.8, 131.1, 130.8, 130.5, 115.1, 86.6, 74.6, 71.5, 65.9, 33.4, 29.3, 28.1, 26.0, 25.3, 18.4, 18.3, -4.3, -4.7; IR (film) ν_{max} 2955, 2930, 2856, 2238, 1713, 1249, 1072; ESIMS *m/z* 399 ([M + Na^+], $C_{22}H_{36}NaO_3Si$ requires 399). HRMS (FAB$^+$) *m/z* 375.2363 ([M - H]$^+$, $C_{22}H_{35}O_3Si$ requires 375.2355).

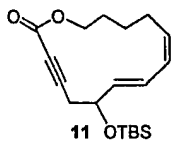
To a solution of the above alkynoic ester (77.8 mg, 0.207 mmol) in toluene (9 mL) was added $Co_2(CO)_8$ (99.0 mg, 0.289 mmol). The mixture was stirred at room temperature for 45 min, and then concentrated in vacuum. The dark residue was purified by PTLC (15:1 hexane/EtOAc) to give cobalt complex **9** (117.5 mg, 86%) as a red oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.17 (dd, J = 15.3, 10.6 Hz, 1H), 6.03 (ddd, J = 15.3, 11.3 Hz, 1H), 5.68 (dd, J = 14.9, 6.9 Hz, 1H), 5.61 (dd, J = 15.2, 6.8 Hz, 1H), 5.38 (ddd, J = 17.1, 10.1, 8.7 Hz, 1H), 5.10 (q, J = 6.4 Hz, 1H), 5.04 (dd, J = 17.1, 1.3 Hz, 1H), 4.85 (dd, J = 10.3, 1.4 Hz, 1H), 4.41, (m, 1H), 3.20-3.15 (m, 2H), 1.76 (d, J = 6.7 Hz, 3H), 1.59 (t, J = 6.6 Hz, 2H), 1.32 (d, J = 6.2 Hz, 3H), 1. 22-1.17 (m, 1H), 0.90 (s, 9H), 0.82-0.72 (m, 1H), 0.66-0.59 (m, 2H), 0.09, 0.08 (2s, 6H); ^{13}C NMR ($CDCl_3$, 100 Hz) δ 198.7, 169.2, 141.4, 134.3, 132.5, 132.4, 132.2, 131.0, 130.2, 128.8, 127.2, 127.1, 112.0, 93.0, 81.0, 73.7, 73.6, 73.1, 40.0, 26.1, 22.4, 19.9, 18.6, 18.4, 17.4, 13.9, 13.5, -4.2, -4.3, -4.6; IR (film) ν_{max} 2956, 2930, 2858, 2097, 2058, 2029, 1703, 1221, 1065; ESIMS *m/z* 685 ([M + Na^+], $C_{28}H_{36}Co_2NaO_9Si$ requires 685).



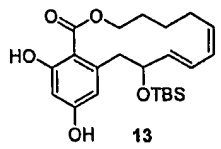
10

RCM product **10**. To a solution of cobalt complex **9** (16 mg, 0.024 mmol) in dry CH_2Cl_2 (120 mL) was added tricyclohexyl phosphine[1,3-bis(2,4,6-trimethylphenyl)-

4,5-dihydroimidazol-2-ylidene]-[bezyli-dene] ruthenium(IV) dichloride (second generation Grubbs catalyst) (6.1 mg, 0.0072 mmol). The resulting solution was heated to 45 °C for 1 hr and 10 min, then cooled to room temperature and filtered through a plug of silica gel. The solvent was removed under reduced pressure. The residue was purified by PTLC (15:1 hexane/EtOAc) to give cyclic product **10** (10.5 mg, 71%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.44 (dd, J = 15.3, 10.8 Hz, 1H), 5.97 (t, J = 10.8 Hz, 1H), 5.58 (dd, J = 15.4, 7.5 Hz, 1H), 5.54 (dt, J = 10.1, 4.5 Hz, 1H), 4.57-4.47 (m, 2H), 4.25-4.20 (m, 1H), 3.45-3.36 (m, 2H), 2.45-2.37 (m, 1H), 2.13-1.05 (m, 1H), 1.99-1.79 (m, 1H), 1.80-1.62 (m, 2H), 1.51-1.41 (m, 1H), 0.92 (s, 9H), 0.11, 0.08 (2s, 6H); ^{13}C NMR ($CDCl_3$, 100 Hz) δ 199.9, 170.4, 135.2, 132.7, 129.0, 127.1, 92.8, 77.4, 73.1, 65.0, 45.2, 26.4, 26.1, 25.4, 18.5, 1.2, -4.2, -4.6; IR (film) ν_{max} 2955, 2930, 2857, 2098, 2059, 2027, 1702, 1213, 1057; ESIMS *m/z* 643 ([M + Na^+], $C_{25}H_{30}Co_2NaO_9Si$ requires 643).

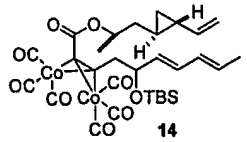


Model cyclic alkyne **11**. To a solution of compound **10** (35.6 mg, 0.0574 mmol) in acetone at -10 °C was added ammonium cerium (IV) nitrate (189 mg, 0.344 mmol) portionwise. After 10 min at -10 °C, the reaction was quenched by addition of diisopropylethylamine (0.18 mL, 1.03 mmol). The resulting mixture was filtered through a plug of neutral alumina, and the solvent was removed under reduced pressure. Purification by PTLC (15:1 hexane/EtOAc) afforded cyclic alkyne **11** (17.6 mg, 92%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.64 (dd, J = 15.5, 11.1 Hz, 1H), 6.07 (t, J = 11.0 Hz, 1H), 5.53 (dd, J = 15.5, 7.1 Hz, 1H), 5.40 (dt, J = 10.4, 4.8 Hz, 1H),), 4.41-4.25 (m, 2H), 4.06-4.01 (m, 1H), 2.69-2.62 (m, 1H), 2.56 (dd, J = 17.1, 4.4 Hz, 1H), 2.46 (dd, J = 17.1, 9.5 Hz, 1H), 2.26-2.21 (m, 1H), 1.75-1.61 (m, 4H), 0.89 (s, 9H), 0.08, 0.07 (2s, 6H); ^{13}C NMR ($CDCl_3$, 100 Hz) δ 153.9, 133.2, 132.9, 128.5, 128.2, 87.7, 77.0, 72.9, 68.0, 29.0, 28.2, 26.7, 26.0, 25.6, 18.3, -4.3, -4.7; IR (film) ν_{max} 2954, 2929, 2857, 2238, 1716, 1245, 1110, 1075, 837; ESIMS *m/z* 357 ([M + Na^+], $C_{19}H_{30}NaO_3Si$ requires 357). HRMS(FAB$^+$) *m/z* 333.1888 ([M - H]$^+$, $C_{19}H_{29}O_3Si$ requires 333.1886).



13

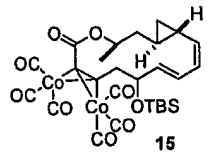
Diels-Alder product **13**. Cyclic alkyne **11** (27 mg, 0.081 mmol) and excess diene **12** (0.30 mL, 0.90 mmol) were mixed and heated in a sealed vial to 140 °C for 48.5 hours. The mixture was cooled to room temperature, loaded onto a PTLC plate, and purified (4:1 hexane/EtOAc) to afford aromatic product **13** (20 mg, 60%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 11.64, (s, 1H), 6.38 (dd, J = 15.4, 10.9 Hz, 1H), 6.35 (d, J = 2.6 Hz, 1H), 6.30 (d, J = 2.6 Hz, 1H), 6.23 (t, J = 10.6 Hz, 1H), 5.95 (s, 1H), 5.78 (dd, J = 15.3, 8.4 Hz, 1H), 5.60 (q, J = 9.9 Hz, 1H), 4.69 (q, J = 9.1 Hz, 1H), 4.12 (t, J = 8.5 Hz, 2H), 3.63 (d, J = 13.0 Hz, 1H), 2.62 (dd, J = 13.1, 8.9 Hz, 1H), 2.50-2.40 (m, 1H), 2.12-2.05 (m, 1H), 1.87-1.76 (m, 2H), 1.56-1.44 (m, 2H), 0.78 (s, 9H), -0.20, -0.25 (2s, 6H); ^{13}C NMR ($CDCl_3$, 100 Hz) δ 172.2, 165.7, 160.3, 144.8, 135.4, 131.0, 129.9, 126.0, 113.0, 105.3, 102.2, 78.7, 64.3, 46.6, 25.9, 24.2, 23.7, 23.0, 18.4, -4.7, -5.0; IR (film) ν_{max} 3380, 2954, 2929, 2856, 1648, 1620, 1254, 1169, 1106, 1061, 837; ESIMS *m/z* 441 ([M + Na^+], $C_{23}H_{34}NaO_5Si$ requires 441). HRMS (FAB^+) *m/z* 418.2173 ($[M]^+$, $C_{23}H_{34}O_5Si$ requires 418.2176).



14

Cobalt complex **14**. To a solution of alkyne **8** (526 mg, 1.31 mmol) in toluene (60 mL) was added $Co_2(CO)_8$ (625 mg, 1.83 mmol). The mixture was stirred at room temperature for 30 min, and the solvent was removed under reduced pressure. The dark residue was purified by flash chromatography (silica, 0 to 5% EtOAc in hexane) to give cobalt complex **14** (902 mg, 100%) as an inseparable mixture of two diastereomers. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.17 (dd, J = 15.3, 10.6 Hz, 1H), 6.03 (ddd, J = 15.3, 11.3 Hz, 1H), 5.68 (dd, J = 14.9, 6.9 Hz, 1H), 5.61 (dd, J = 15.2, 6.8 Hz, 1H), 5.38 (ddd, J = 17.1, 10.1, 8.7 Hz, 1H), 5.10 (q, J = 6.4 Hz, 1H), 5.04 (dd, J = 17.1, 1.3 Hz, 1H), 4.85 (dd, J = 10.3, 1.4 Hz, 1H), 4.41, (m, 1H), 3.20-3.15 (m, 2H), 1.76 (d, J = 6.7 Hz, 3H), 1.59 (t, J = 6.6 Hz, 2H), 1.32 (d, J = 6.2 Hz, 3H), 1. 22-1.17 (m, 1H), 0.90 (s, 9H),

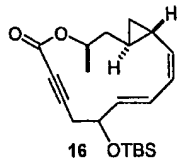
0.82-0.72 (m, 1H), 0.66-0.59 (m, 2H), 0.09, 0.08 (2s, 6H); ^{13}C NMR ($CDCl_3$, 100 Hz) δ 198.7, 169.2, 141.4, 134.3, 132.5, 132.4, 132.2, 131.0, 130.2, 128.8, 127.2, 127.1, 112.0, 93.0, 81.0, 73.7, 73.6, 73.1, 40.0, 26.1, 22.4, 19.9, 18.6, 18.4, 17.4, 13.9, 13.5, -4.2, -4.3, -4.6; IR (film) ν_{max} 2956, 2930, 2858, 2097, 2058, 2029, 1703, 1221, 1065; ESIMS *m/z* 711 ([M + Na$^+$], $C_{30}H_{38}Co_2NaO_9Si$ requires 711).



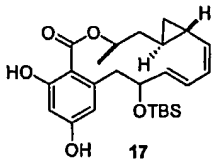
15

RCM product **15**. To a solution of alkyne-cobalt complex **14** (67 mg, 0.097 mmol) in dry CH_2Cl_2 (485 mL) was added tricyclohexyl phosphine[1,3-bis(2,4,6-trimethylphenyl)-4,5-dihydroimidazol-2-ylidene] [bezylidene] ruthenim(IV) dichloride (second generation Grubbs catalyst) (21 mg, 0.025 mmol). The resulting mixture was heated to 45 °C for 1.5 hours, and filtered through a short column of silica gel. The filtrate was concentrated in vacuum. The residue was purified by PTLC (15:1 hexanes/EtOAc) to give cyclic product **15** as a 2:1 mixture of two separable diastereomers. Major isomer (23.1 mg, 37%): ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.49 (dd, *J* = 15.4, 10.9 Hz, 1H), 5.84 (t, *J* = 10.6 Hz, 1H), 5.50 (dd, *J* = 15.4, 8.5 Hz, 1H), 5.06 (dd, *J* = 10.5, 6.9 Hz, 1H), 5.02-4.94 (m, 1H), 4.81-4.75 (m, 1H). 3.48-3.36 (m, 2H), 2.22-2.25 (m, 1H), 1.58-1.50 (m, 1H), 1.50-1.46 (m, 1H), 1.32 (d, *J* = 6.2 Hz, 3H), 0.91 (s, 9H), 0.90-0.80 (m, 1H), 0.89 (s, 9H), 0.67-0.63 (m, 1H), 0.59-0.55 (m, 1H), 0.12, 0.09 (2s, 6H); ^{13}C NMR ($CDCl_3$, 100 Hz) δ 199.0, 170.4, 135.7, 134.9, 129.2, 127.8, 92.0, 77.5, 72.5, 72.1, 45.5, 38.0, 26.1, 10.3, 18.4, 16.4, 16.0, -4.2, -4.6; IR (film) ν_{max} 2955, 2928, 2854, 2097, 2060, 2029, 1692, 1232, 1056; ESIMS *m/z* 669 ([M + Na$^+$], $C_{27}H_{32}Co_2NaO_9Si$ requires 669); $[\alpha]^{25}_D$ -127 (*c* 0.11, $CHCl_3$). Minor isomer (12.6 mg, 20%): ^{1}H NMR ($CDCl_3$, 500 MHz) δ 6.60 (dd, *J* = 15.7, 9.9 Hz, 1H), 5.86 (t, *J* = 10.3 Hz, 1H), 5.81 (dd, *J* = 15.7, 4.4 Hz, 1H), 5.12-5.05 (m, 1H), 4.99 (t, *J* = 9.9 Hz, 1H), 4.46-4.44 (m, 1H), 3.58 (dd, *J* = 15.3, 9.4 Hz, 1H), 3.32 (dd, *J* = 15.3, 4.2 Hz, 1H), 2.16 (dt, *J* = 15.3, 4.2 Hz, 1H), 1.65-1.59 (m, 2H), 1.36 (d, *J* = 6.5 Hz, 3H), 0.94 (s, 9H), 0.64-0.59 (m, 2H), 0.14 (s, 6H); ^{13}C NMR ($CDCl_3$, 125 Hz) δ 198.6, 169.7, 135.9, 132.6, 126.8, 126.5, 92.0, 80.7, 73.4, 73.0, 41.8, 37.7, 26.1, 19.3, 18.5, 18.4, 15.1, 13.7,

-4.5, -4.6; IR (film) ν_{max} 2929, 2856, 2097, 2059, 2028, 1702, 1220, 1059; ESIMS *m/z* 669 ([M + Na^+], $C_{27}H_{32}Co_2NaO_9Si$ requires 669). $[\alpha]^{25}_D$ +48 (*c* 0.19, $CHCl_3$).

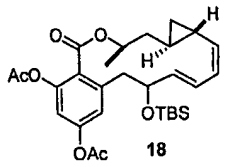


Cyclic alkyne **16**. The major isomer of **15** (23.1 mg, 0.0358 mmol) was dissolved in dry THF (1 mL). At 0 °C, a solution of I_2 (135 mg, 0.536 mmol) in THF (5 mL) was added. After 35 minutes at 0 °C, the reaction was quenched by the addition of a 2 mL 1:1 mixture of sat. aqueous $Na_2S_2O_3$ and $NaHCO_3$, followed by warming to room temperature and diluting with EtOAc (20 mL). The layers were separated, and the organic layer was washed with sat. aqueous NH_4Cl, dried (Na_2SO_4), filtered and concentrated. The residue was purified by PTLC (15:1, hexanes/EtOAc) to cyclic alkyne **16** as colorless oil (8.9 mg, 69%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.70 (dd, *J* = 15.6, 11.1 Hz, 1H), 5.98 (t, *J* = 10.8 Hz, 1H), 5.51 (dd, *J* = 15.6, 7.7 Hz, 1H), 5.20-5.14 (m, 1H), 4.92 (t, *J* = 10.7 Hz, 1H), 4.38-4.33 (m, 1H), 2.59 (dd, *J* = 16.8, 4.3 Hz, 1H), 2.41 (dd, *J* = 16.7, 10.9 Hz, 1H), 2.07 (dt, *J* = 14.8, 1.8 Hz, 1H), 1.74-1.67 (m, 1H), 1.33 (d, *J* = 6.5 Hz, 3H), 1.19-1.12 (m, 1H), 0.93-0.87 (m, 1H), 0.89 (s, 9H), 0.69-0.65 (m, 1H), 0.63-0.58 (m, 1H), 0.07 (s, 6H); ^{13}C NMR ($CDCl_3$, 100 Hz) δ 153.4, 136.5, 132.5, 128.3, 125.4, 85.7, 77.4, 73.8, 72.0, 38.6, 29.2, 26.0, 20.4, 18.4, 18.2, 14.6, 12.8; IR (film) ν_{max} 2954, 2928, 2856, 2238, 1706, 1252, 1105, 1072, 1004; ESIMS *m/z* 383 ([M + Na^+], $C_{21}H_{32}NaO_3Si$ requires 383); HRMS (FAB^+) *m/z* 360.2130 ([M]$^+$, $C_{21}H_{32}O_3Si$ requires 360.2121). $[\alpha]^{25}_D$ +56 (*c* 0.95, $CHCl_3$).



Diels-Alder product **17**. Compound **16** (156 mg, 0.43 mmol) was dissolved in diene **12** (0.75 mL, 2.3 mmol) and heated to 140 °C in a sealed vial for 66 hours. The reaction mixture was cooled to room temperature and purified by PTLC (4:1, hexanes/EtOAc) to give **17** (143 mg, 75%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 11.58 (s, 1H), 6.56 (dd, *J* =

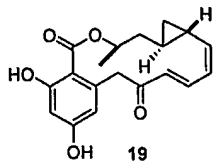
15.9, 9.3 Hz, 1H), 6.38 (d, J = 2.4 Hz, 1H), 6.33 (d, J = 2.4 Hz, 1H), 5.94 (t, J = 9.5 Hz, 1H), 5.68 (dd, J = 15.9, 6.8 Hz, 1H), 5.46-5.43 (m, 1H), 5.34 (dd, J = 10.1, 4.3 Hz, 1H), 4.51 (q, J = 6.7 Hz, 1H), 3.79 (dd, J = 13.6, 6.2 Hz, 1H), 2.81 (dd, J = 13.1, 8.9 Hz, 1H), 1.99-1.81 (m, 2H), 1.44 (d, J = 6.5 Hz, 3H), 1.21-1.13 (m, 1H), 0.93-0.87 (m, 1H), 0.88 (s, 9H), 0.58-0.51 (m, 2H), -0.02 (s, 6H); ^{13}C NMR ($CDCl_3$, 100 Hz) δ 164.8, 160.5, 143.8, 134.8, 133.7, 129.6, 128.0, 112.3, 106.1, 102.1, 75.7, 72.9, 42.6, 38.0, 26.1, 18.7, 18.4, 16.4, 16.1, 14.8, 1.4, -4.4, -4.6; IR (film) ν_{max} 3376, 2954, 2928, 2856, 1644, 1619, 1257, 1062, 835; ESIMS *m/z* 467 ([M + Na^+], $C_{25}H_{36}NaO_5Si$ requires 467). HRMS (FAB^+) *m/z* 444.2336 ([M]$^+$, $C_{25}H_{36}O_5Si$ requires 444.2332). $[\alpha]^{25}_D$ +3.1 (*c* 1.3, $CHCl_3$).



18

To a solution of **17** (243 mg, 0.546 mmol) in anhydrous DMF (13.5 mL) was added acetic anhydride (2.9 mL) and 4-(dimethylamino)pyridine (12.0 mg, 0.0546 mmol) sequentially. After 30 min at room temperature, the reaction was quenched by addition of 50 mL of pH 7.2 phosphate buffer. The resulting mixture was diluted with EtOAc (75 mL), separated and the aqueous layer was extracted with additional EtOAc (2 x 50 mL). The combined organic layers were washed with 5% aqueous NaCl (2 x 45 mL). The combined washings were extracted with EtOAc (2 x 30 mL). The combined organic layers were washed with sat. aqueous NaCl (30 mL), dried (Na_2SO_4), filtered and concentrated. Purification of the residue by PTLC (4:1 hexane/EtOAc) gave diacetate **18** (250 mg, 87%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.96 (d, J = 2.1 Hz, 1H), 6.88 (d, J = 2.1 Hz, 1H), 6.59 (dd, J = 16.0, 10.5 Hz, 1H), 5.89 (t, J = 10.5 Hz, 1H), 5.66 (dd, J = 16.1, 4.9 Hz, 1H), 5.26-5.23 (m, 2H), 4.51 (q, J = 5.8 Hz, 1H), 3.12-3.02 (m, 2H), 2.28, 2.23 (2s, 6H), 1.47 (d, J = 6.2 Hz, 3H), 1.46-1.39 (m, 1H), 1.14 (ddd, J = 14.9, 10.1, 2.0 Hz, 1H), 0.96-0.87 (m, 1H), 0.87 (s, 9H), 0.52-0.48 (m, 2H), -0.01, -0.02 (2s, 6H); ^{13}C NMR ($CDCl_3$, 100 Hz) δ 168.6, 168.4, 166.4, 151.4, 148.7, 139.1, 135.9, 133.6, 129.2, 127.2, 125.6, 120.4, 114.7, 73.8, 73.2, 43.2, 40.0, 26.0, 21.3, 21.0, 19.3, 18.3, 16.9, 16.2, 17.7, -4.6, -4.7; IR (film) ν_{max} 2954, 2928, 2856, 1775, 1720, 1612,

1191, 1134, 1069; ESIMS *m/z* 551 ([M + Na^+], $C_{29}H_{40}NaO_7Si$ requires 551); HRMS (FAB^+) *m/z* 528.2569 ([M]$^+$, $C_{29}H_{40}O_7Si$ requires 528.2543). $[\alpha]^{25}_D$ -38 (*c* 1.1, $CHCl_3$).

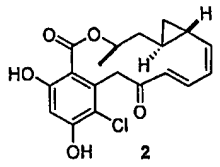


19

Cyclopropamonocillin **19**. To a solution of diacetate **18** (250 mg, 0.473 mmol) in THF (10.2 mL) at 0 °C was added pyridine (3.4 mL) and HF-Pyridine complex (1.7 mL) sequentially. The resulting mixture was stirred at room temperature for 10.5 hrs. TMSOMe (30 mL) was added, and stirring was continued for 45 min to quench the remaining HF. The solvents were removed under reduced pressure. The alcohol isolated was dried under high vacuum, and dissolved in CH_2Cl_2 (15 mL) and cooled to 0 °C. Dess-Martin periodinane (301 mg, 0.710 mmol) was added, and the resulting mixture was stirred at room temperature for 15 min. The solution was then directly loaded on PTLC plates and purified (1:1 hexane/EtOAc) to give the desired ketone (133 mg, 68%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.01 (dd, *J* = 16.1, 11.3 Hz, 1H), 6.97 (d, *J* = 1.4 Hz, 1H), 6.92 (d, *J* = 2.0 Hz, 1H), 6.20 (t, *J* = 10.4 Hz, 1H), 5.96 (d, *J* = 16.0 Hz, 1H), 5.60 (dd, *J* = 10.0, 7.2 Hz, 1H), 5.47-5.41 (m, 1H), 4.20 (d, *J* = 13.8 Hz, 1H), 3.77 (d, *J* = 13.8 Hz, 1H), 2.31 (dt, *J* = 15.3, 4.4 Hz, 1H), 2.25, 2.24 (2s, 6H), 1.73-1.71 (m, 1H), 1.50 (d, *J* = 6.5 Hz, 3H), 1.26-1.21 (m, 1H), 1.00-0.97 (m, 1H), 0.75-0.69 (m, 2H); ^{13}C NMR ($CDCl_3$, 125 Hz) δ 198.4, 168.5, 165.0, 152.1, 149.2, 145.6, 143.7, 135.7, 129.6, 128.8, 124.6, 119.1, 115.6, 72.8, 43.0, 38.3, 21.2, 18.2, 16.7, 16.5, 15.6; IR (film) ν_{max} 2928, 1774, 1728, 1657, 1621, 1586, 1290, 1190, 1132, 1029; ESIMS *m/z* 435 ([M + Na^+], $C_{23}H_{24}NaO_7$ requires 435); HRMS (FAB^+) *m/z* 413.1616 ([M + H]$^+$, $C_{23}H_{25}O_7$ requires 413.1600). $[\alpha]^{25}_D$ -269 (*c* 1.43, $CHCl_3$).

The above ketone (169 mg, 0.409 mmol) was dissolved in 26 mL 1:1 MeOH and 5% aqueous $NaHCO_3$, and stirred at room temperature for 14 hrs to remove the phenolic acetates. The resulting solution was diluted with sat. aqueous NH_4Cl (80 mL) and EtOAc (100 mL) and separated. The aqueous layer was extracted with additional EtOAc (3 x 75 mL). The combined organic layers were dried (Na_2SO_4), filtered and concentrated in vacuum. Purification by PTLC (1:1 hexane/EtOAc) provided **19** (124

mg, 92%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 11.33 (s, 1H), 8.36 (dd, J = 16.1, 11.7 Hz, 1H), 6.48 (d, J = 1.6 Hz, 1H), 6.40 (d, J = 2.5 Hz, 1H), 6.20 (t, J = 11.3 Hz, 1H), 6.01 (d, J = 15.9 Hz, 1H), 5.72 (dd, J = 9.8, 6.2 Hz, 1H), 5.49-5.45 (m, 1H), 5.32 (d, J = 13.6 Hz, 1H), 3.51 (d, J = 13.7 Hz, 1H), 2.31 (dt, J = 15.8, 3.3 Hz, 1H), 1.61-1.55 (m, 1H), 1.55 (d, J = 6.7 Hz, 3H), 1.32-1.28 (m, 1H), 1.03-0.97 (m, 1H), 0.74-0.72 (m, 1H), 0.64-0.63 (m, 1H); ^{13}C NMR ($CDCl_3$, 125 Hz) δ 201.8, 170.1, 165.6, 161.6, 145.3, 139.2, 129.5, 128.5, 109.4, 104.5, 102.7, 73.5, 43.2, 27.6, 17.9, 17.2, 15.9, 13.9; IR (film) ν_{max} 3260, 1650, 1618, 1586, 1447, 1259, 1160, 1099, 996, 854; ESIMS *m/z* 351 ([M + Na^+], $C_{19}H_{20}NaO_5$ requires 351); HRMS (FAB$^+$) *m/z* 329.1382 ([M + H]$^+$, $C_{19}H_{21}O_5$ requires 329.1389). (+)-(*R, R, S*)-**19**: $[\alpha]^{25}_D$ -189 (*c* 0.77, CH_2Cl_2).



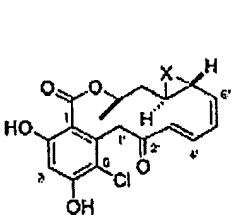
2

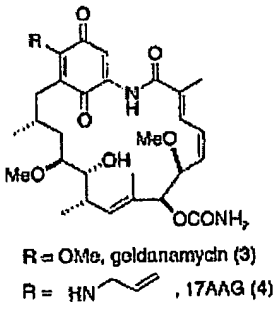
Cyclopropa**r**adicicol **2**. Compound **19** (26.5 mg, 0.081 mmol) was dissolved in dry CH_2Cl_2 (5 mL) and cooled to 0 °C. A solution of SO_2Cl_2 in CH_2Cl_2 (4.5 mL, diluted from 0.123 mL 1 M solution in CH_2Cl_2, 0.123 mmol) was added dropwise. After 45 minutes, the reaction was quenched by the addition of 5 mL of 5% NH_4Cl, and diluted with CH_2Cl_2. The layers were separated, and the organic phase was washed with brine, dried ($MgSO_4$), filtered and concentrated in vacuum. Purification of the residue by PTLC (3:1, hexanes/EtOAc) gave **2** as a white solid (17.8 mg, 61%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 10.89 (s, 1H), 8.00 (dd, J = 15.9, 11.3 Hz, 1H), 6.63 (s, 1H), 6.42 (s, 1H), 6.13 (t, J = 11.2 Hz, 1H), 6.04 (d, J = 16.3 Hz, 1H), 5.62 (dd, J = 9.9, 5.8 Hz, 1H), 5.46-5.42 (m, 1H), 4.90 (broad d, J = 15.9 Hz, 1H), 3.77 (bs, 1H), 2.22 (dt, J = 15.8, 3.2 Hz, 1H), 1.60-1.52 (m, 1H), 1.48 (d, J = 6.7 Hz, 3H), 1.15-1.11 (m, 1H), 0.90-0.85 (m, 1H), 0.69-0.65 (m, 1H), 0.57-0.54 (m, 1H); ^{13}C NMR ($CDCl_3$, 125 Hz) δ 199.2, 169.4, 162.7, 155.6, 143.5, 142.5, 136.9, 129.9, 128.9, 115.6, 107.8, 103.5, 74.4, 46.4, 37.4, 17.9, 17.3, 15.6, 13.6; IR (film) ν_{max} 3341, 1716, 1651, 1609, 1578 cm^{-1}; ESIMS *m/z* 385 ([M + Na^+], $C_{19}H_{19}NaO_5Cl$ requires 385); HRMS (ESI) *m/z* 385.0820 ([M + Na^+], $C_{19}H_{19}NaO_5Cl$ requires 385.0819). (+)-(*R, R, S*)-**2**: $[\alpha]^{25}_D$ +69 (*c* 0.87, CH_2Cl_2).

Example 3-Synthesis of Resorcinylic Macrolides via Ynolides: Establishment of Cycloproparadicicol as Synthetically Feasible Preclinical Anticancer Agent Based on Hsp90 as the Target

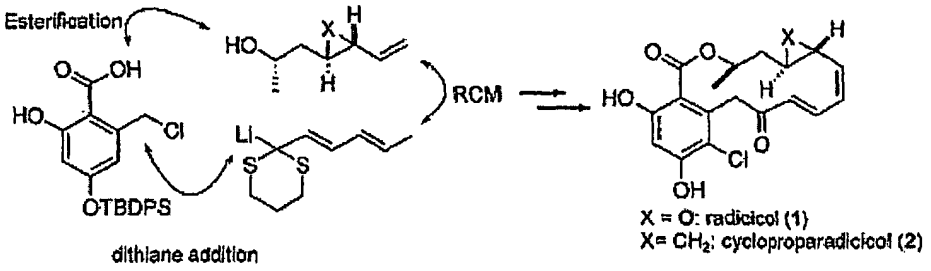
Introduction

The heat shock protein 90 (Hsp90) is a molecular chaperone that mediates the stabilization and folding of various oncogenic proteins, such as *Raf1* and *Her2* (Banerji, U.; Judson, I.; Workman, P. *Curr. Cancer Drug Targets* 2003, 3, 385-390; incorporated herein by reference) Recently, Hsp90 has attracted extensive attention as a novel, potential antitumor target. The natural product family which has attracted by far the greatest attention as a potential inhibitor of the action of Hsp90 are congeners of geldanamycin (**3**) (Delmotte, P.; Delmotte-Plaquee, *J. Nature* 1953, 171, 344; Ayer, W. A.; Lee, S. P.; Tsuneda, A.; Hiratsuka, *Y. Can. J. Microbiol.* 1980, 26, 766-773; Roe, S. M.; Prodromou, C.; O'Brien, R.; Ladbury, J. E.; Piper, P. W.; Pearl, L. H. *J. Med. Chem.* 1999, 42, 260-266; each of which is incorporated herein by reference) Indeed, a derivative of geldanamycin, 17AAG (**4**), is the most advanced of drug candidates based on Hsp90 and is currently in phase II clinical trials (below). (Banerji, U.; Judson, I.; Workman, *P. Curr. Cancer Drug Targets* 2003, 3, 385-390; DeBoer, C.; Meulman, P. A.; Wnuk, R. J.; Peterson, D. H. *J. Antibiot.* 1970, 23, 442-447; each of which is incorporated herein by reference)





Structures of Hsp90 inhibitors.



Scheme 3-1. Our First Total Synthesis of Cycloproparadicicol

Though our laboratory has been involved in attempting to build upon 17AAG as a discovery lead centering around Hsp90 (Kuduk *et al. Bioorg. Med Chem Len.* 2000,10, 1303-1306; Zheng *et al. Cancer Res.* 2000, 60, 2090-2094; Kuduk *et al. Bioorg. Med Chem. Lett.* 1999, 9, 1233-1238; Chiosis *et al. Curr. Cancer Drug Targets* 2003, 3, 371-376; each of which is incorporated herein by reference), we hoped to evaluate the potentialities of another natural product targeting Hsp90, *i.e.*, radicicol. We postulated that radicicol-derived drug candidates could in the long run out perform "geldanamycinoids" in that the latter would carry potential liabilities from the ansa bridged quinone substructure. Indeed, in a potentially important comparison, radicicol is significantly less hepatotoxic than 17AAG (Chiosis *et al. Curr. Cancer Drug Targets* 2003, 3, 371-376; incorporated herein by reference). However, radicicol was found to be ineffective in vivo animal models. We theorized that the epoxide both in radicicol and in radicicol oxime (which is active in in vivo models (Agatsuma *et al. Bioorg. Med. Chem.* 2002, 10, 3445-3454; incorporated herein by reference) could well be a source of nonspecific cytotoxicity, which could narrow the exploitable margin of therapeutic index. Furthermore, the potential chemical vulnerability of the dienyl epoxide raised concerns about drug shelf stability as well as pharmacokinetics. With a view to molecular editing, of the oxido function in a setting of minimal conformational perturbation of the radicicol lead, we were drawn to analogue **2** in which the epoxide linkage is replaced by a cyclopropane.

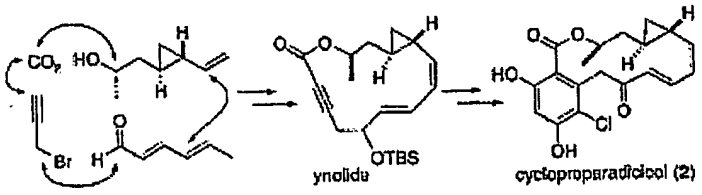
We had previously shown that compound **2** has an in vitro biological profile comparable to that of **1** (Yamamoto *et al. Angew. Chem., Int. Ed.* 2003, 42, 1280-1284; incorporated herein by reference). Moreover, we brought to bear an important line of evidence that **2** and **1** were closely related in their interactions with their biotargets. Thus changes in peripheral stereogenic centers in both **1** and **2** bring about the same

consequences in biological function. In other words, both structures as shown are optimized from a stereo-chemical perspective. Moreover, and of considerable potential advantage for radicicol-based inhibitors, they also display cytotoxicity against Rb (retinoblastoma)-negative cells known to be resistant to 17AAG (Yamamoto *et al. Angew. Chem., Int. Ed.* 2003, 42, 1280-1284; incorporated herein by reference)

These promising findings inevitably raised issues as to the availability of cycloproparadicicol. Clearly, our first synthesis of this compound (Yamamoto *et al. Angew. Chem., Int. Ed.* 2003, 42, 1280- 1284; incorporated herein by reference), while touching on several issues of academic interest in organic chemistry, did not seem promising for producing more than token amounts of the now interesting **2**.

As seen, the first synthesis from our lab relied on the appropriate sequenced Mitsunobu esterification, dithiane alkylation, and ring closing olefin metathesis. While highly convergent and concise, this first generation pathway to radicicol suffered from several low yielding steps which did not improve following attempted optimization. In particular, the low yields associated with the dithiane alkylation and ring-closing metathesis (RCM) steps sharply curtailed access to cycloproparadicicol for evaluation (Yamamoto *et al. Angew. Chem., Int. Ed.* 2003, 42, 1280-1284; incorporated herein by reference)

Indeed, a new strategy has been formulated and reduced to practice, resulting in a much improved second generation total synthesis of cycloproparadicicol (Yang *et al. J. Am. Chem. Soc.* 2003, 125, 9602-9603; incorporated herein by reference). In addition, the new route shows promise of applicability to a broad range of resorcinylic macrolides (Geng *et al. J. Org. Len.* 2004, 6, 413-416; incorporated herein by reference). Herein, we disclose the full details of this new approach and its application to reach cycloproparadicicol as well as aigalomycin D. The synthesis and biological activities of a number of interesting analogues of cycloproparadicicol will be described. Based on the much improved route of synthesis, and based on early investigations of biological profiles, cycloproparadicicol now emerges as a candidate for full scale preclinical evaluation.



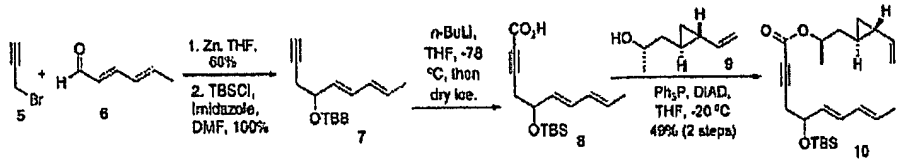
Scheme 3-2. New Ynolide Approach for Cyclopropparadicicol

Results and Discussions

Overall Strategy. The defining element of our second generation strategy was the building of the aromatic sector of the resorcinylic marcrolide by Diels-Alder reaction of a new type of dienophile, *i.e.*, an "ynolide". This cycloaddition route to the benzo-fused macrolactone represents a substantial departure from the usual mode of synthesis in which one starts with an aromatic ring and appends to it suitable arms to close the macrolactone ring (cf. Schemes 3-1 and 3-2). We hoped that the new ynolide approach, if successful, would be highly convergent and would allow for rapid access to a broad family of resorcinylic macrolides. Since it has been our experience that monoactivated acetylenic dienophiles are surprisingly weakly reactive in Diels-Alder reactions (Danishefsky *et al. J. Org. Chem.* 1979, 44, 4716-4717; incorporated herein by reference), the success of a projected DielsAlder cycloaddition aromatization sequence could not have been anticipated with confidence.

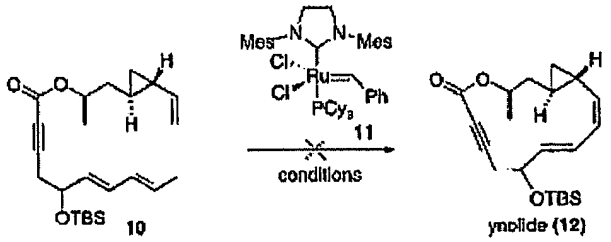
Synthesis of Acyclic Alkynoic Ester. To facilitate progress to the key issues of the plan, we sought to develop an efficient synthesis route to reach a seco-alkynoate ester, which, following RCM, would afford the ynolide dienophile. In practice, the synthesis commenced with commercial 2,4-hexadienal (sorbaldehyde, **6**, Scheme 3-3). Reformatsky-like condensation of propargyl bromide (**5**) with **6** led to the expected carbinol. Following β-esterification, alkyne precursor **7** was in hand in good yield (Friedrich *et al. J. Org. Chem.* 1996, 61, 1082-1100; incorporated herein by reference). Treatment of **7** with *n*-butyllithium generated an alkynide ion, which was carboxylated with dry ice, to provide acid **8**, in very high yield (Fuganti *et al. Tetrahedron: Asymmetry* 1994, 5, 1135-1138; incorporated herein by reference). Following reaction of racemic **8** and the known optically pure and defined alcohol **9** (Garbaccio *et al. J. Am. Chem. Soc.* 2001, 123, 10903-10908; incorporated herein by reference) under Mitsunobu conditions (Romo *et al. J. Am. Chem. Soc.* 1998, 120, 12237-12254;

incorporated herein by reference), ester **10** was obtained as a diastereomeric mixture at the future $C_{2'}$.



Scheme 3-3. Synthesis of the Acyclic Alkynoic Ester

Scheme 3-4. Attempted Ring-Closing Metathesis Reactions of 10

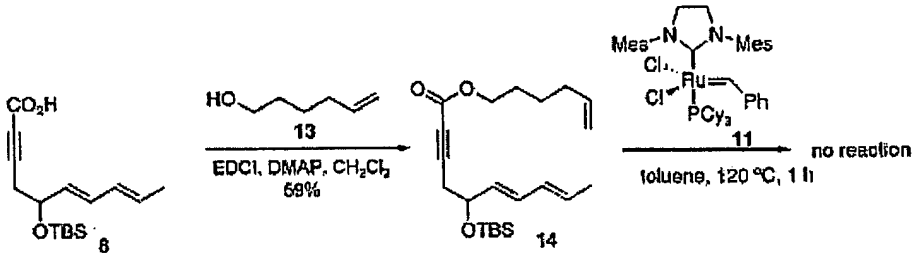


Entry	Conditions	results
1	0.25 equiv 11, CH2Cl2, 40 °C, 0.5 mM, 2 h	recovered 10
2	0.25 equiv 11, toluene, 120 °C, 0.5 mM, 1 h	recovered 10
3	1.1 equiv 11, toluene, 120 °C, 0.2 mM, 10 min	10 decomposed
4	0.3 equiv 11, 0.3 equiv Ti(Oi-Pr)4, CH2Cl2, 0.5 mM, 40 °C, 2 h	recovered 10

Macrocyclization via Ring Closing Olefin Metathesis. The next key reaction was the formation of our hoped macrocyclic ynolide through ring-closing olefin metathesis. For this purpose, we made recourse to the powerful metalo carbene catalysts, innovated by Grubbs and associates, based on ligand **11** (Scholl *et al. Org. Len.* 1999, 1, 953-956; incorporated herein by reference). However, as shown in Scheme 3-4, all efforts to form the desired ynolide **12** using catalytic amounts of **11** provided the recovered starting ester **10**. The Lewis acid, Ti(O*i*-Pr)$_4$, was used in the hope of preventing formation of an inactive complexing locus which included the ester group. However, this attempt at reaction steering was also not successful. Attempted

increase of the amounts of **11** to stoicheometric levels eventually led to decomposition of **10**.

The failure of probe structure **10** to cyclize could be ascribed to conformational rigidities associated with the trans-disubstituted cyclopropane and the linear acetylene "linker." Accordingly, a more flexible model ester **14** was prepared (in 59% yield) using standard protocols. Compound **14** was subjected to RCM conditions (Scheme 3-5). Unfortunately, no formation of cyclic product was observed.



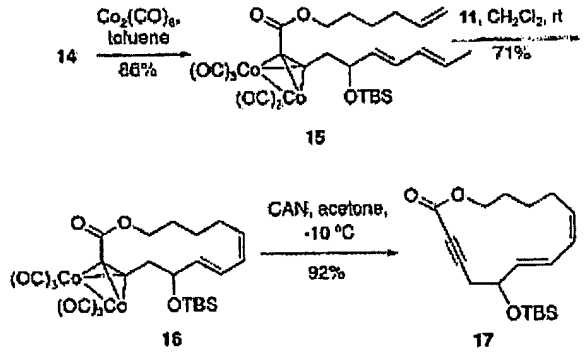
Scheme 3-5. Olefin Metathesis Reaction of Model Ester 14

Cobalt Complexation to Promote RCM. At this stage, we reasoned that the failure of olefin metathesis might be due to the presence of the acetylene group. Aside from the constraint to cyclization imposed by its linear character, the cyclization could further be complicated by nonproductive coordination of the acetylene to the RCM catalytic machinery. The latter possibility could not be dismissed out of hand, since the enyne metathesis reactions are well established (Kinoshita *et al. J. Org. Chem.* 1996, 61, 8356-8357; Mori *et al. J. Org. Chem.* 1998, 63, 6082-6083; incorporated herein by reference). Accordingly, protection of the alkyne could well be helpful. It is well-known that reactions of dicobalt carbonyl with acetylenes can lead to stable complexes, in which the alkyne functions are, in effect, protected (Greenfield *et al. J. Am. Chem. Soc.* 1956, 78, 120-124; Nicholas *et al. Tetrahedron Len.* 1971, 37, 3475-3478; incorporated herein by reference). Moreover, the geometry of cobalt-complexed alkynes optimizes at approximately 140° (Dickson *et al. J. Adv. Organomet. Chem.* 1974, 12, 323-377; incorporated herein by reference). Such a departure from linearity could well favor cyclization (Recently, a similar protocol was reported: Young *et al. J. Org. Chem.* 2003, 68, 3494-3497; incorporated herein by reference). We thus hoped

that cobalt protection of the alkyne could serve to our advantage in the problematic macrocyclization.

To probe the feasibility of this hypothesis, model ester **14** was first treated with cobalt dicarbonyl (Scheme 3-6). Complex formation proceeded readily at room temperature in toluene to provide **15** (in 87% yield) after silica gel chromatography. When complex **15** was subjected to the conditions of ring closing olefin metathesis, with catalyst, cyclization proceeded smoothly at room temperature. The cobalt-protected ynolide **16** was obtained in 71% using 25 mol % of **11** and high dilution (0.2 mM) in methylene chloride.

To retrieve the free acetylene of the ynolide, the cobalt complex had to be "demetalated." This goal was readily achieved by treatment of **16** with excess ceric ammonium nitrate in acetone at low temperature (Magnus, P. *Tetrahedron* 1994, 50, 1397-1418; incorporated herein by reference). Ynolide **17** was obtained in excellent yield (Scheme 6).



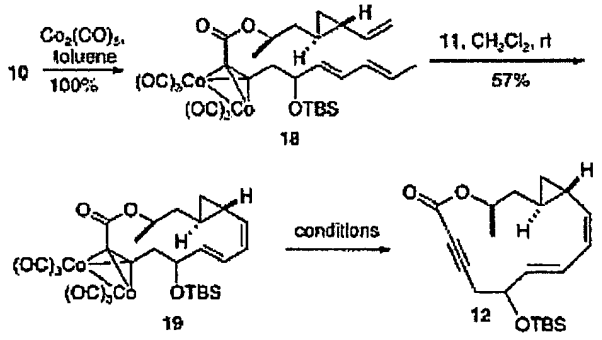
Scheme 3-6. RCM of Cobalt-Protected Model Ester

We next applied this strategy to the target system **10**. Gratifyingly, as was the case with the model compound **15**, RCM reaction of cobalt-complexed **18** proceeded smoothly at room temperature in CH_2Cl_2 to afford cyclic product **19** in 57% yield. Postmetathesis removal of the cobalt, however, was nontrivial. Treatment of **19** with CAN in acetone led primarily to decomposition. Other oxidizing agents such as I_2 (Tanaka *et al. Tetrahedron Len.* 1993, 34, 5757-5760; incorporated herein by reference) or Me_3-NO (Jones *et al. J. Org. Chem.* 1997, 62, 9379-9381; incorporated herein by reference) improved the yields significantly but were not fully reproducible when conducted in large scales. Eventually, it was found that when the solution of **19**

was buffered with 2,6-di-*tert*-butyl pyridine prior to the treatment with CAN, the desired product **12** was isolated in 50% yield in two steps from **18** (Scheme 3-7) (Magnus *et al. J. Am. Chem. Soc.* 1997, 119, 5591-5605; incorporated herein by reference). At this stage, the two stereoisomers of **12** became separable, and they were advanced individually.

Cycloaddition-Aromatization Reactions. Having achieved an efficient synthesis of the required ynolide, we directed our attentions to the next challenge, *i.e.*, the fashioning of the desired resorcinylic macrolides using a Diels-Alder elimination sequence. We appreciated that acetylenic dienophiles, where only one of the sp carbons is activated with a typical activating group such as an ester can be rather unreactive (Danishefsky *et al. J. Org. Chem.* 1979, 44, 4716-4717; incorporated herein by reference). These monoactivated acetylenes must be clearly distinguished at the planning level from diactivated acetylenes such as diesters of acetylenedicarboxylic acid which are powerful dienophiles.

Scheme 3-7. Synthesis of Ynolide 12

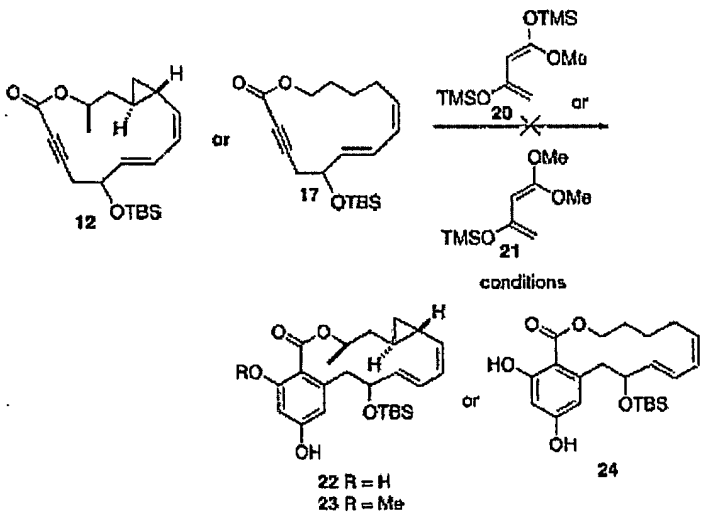


Entry	Conditions	Yields
1	CAN, acetone, -10 °C	<10%
2	I2, THF, 0 °C	< 69%
3	Me,NO, acetone/THF -78 °C tort	66%
4	CAN, DTBP, acetone, -10 °C	50% from 18

At first, trioxygen-substituted dienes of the type **20** (Yamamoto *et al. Chem. Lett* 1978, 649-652; incorporated herein by reference) and **21** (Danishefsky *et al. J. Org. Chem.* 1979, 44, 4716-4717; Banville *et al. J. Chem. Soc., Perkin Trans.* 1 1976,

1852-1856; Danishefsky *et al. J. Org. Chem.* 1978, 43, 379-380; incorporated herein by reference) seemed to be appropriate choices to attempt cycloaddition with ynolides **12**. Ideally, reaction of **12** with diene **20** followed by elimination of MeOH would, upon desilylation, lead to the desired free phenol product **22**. The use of diene **21** would require an additional step to deprotect the phenolic methyl ether of the product **23** (Scheme 3-8). Such deprotections had been found to be nontrivial in related systems (Garbaccio *et al. J. Am. Chem. Soc.* 2001, 123, 10903-10908; incorporated herein by reference).

Scheme 3-8. Attempted Diels—Alder Reactions with Acyclic Dienes OTMS

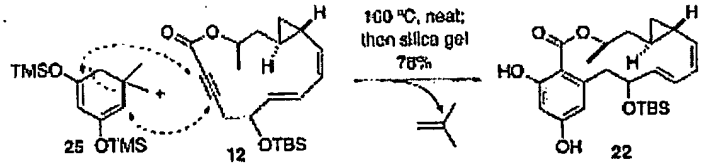


Entry	Conditions	Results
1	**12**, **20**, neat, 75 °C; then Et3N•HF, EtOH	recovered desilylated **12**
2	**12**, **21**, neat, 160 °C; then 0.1 N HCl	Decomposition
3	**17**, **20**, EuFOD, neat, 70 °C; then Et3N•HF, EtOH	recovered desilylated **17**
4	**17**, **20**, Ti(Oi-Pr)4, neat, 70 °C	recovered **17**

To our surprise, when **12** and diene **20** were heated to 70 °C, no aromatic products were isolated. Desilylated **12** was recovered. Since **20** is prone to undergo a 1,5-silyl shift at temperatures above 70 °C (Dickson, R. S.; Fraser, P. *J. Adv. Organomet. Chem.* 1974, 12, 323-377; incorporated herein by reference), we directed our efforts to the thermally more stable diene **21**. Unfortunately, even when **12** and

diene **21** were heated to 160 °C, no products were isolated. The ynolide **12** had decomposed. In the hope of lowering the temperature required for the cycloaddition, we turned to the use of Lewis acids. Considering the extreme acidic liability of these dienes, we chose mild reagents Eufod (Danishefsky *et al. J. Org. Chem.* 1984, 49, 392-393; Castellino *et al. Tetrahedron Len.* 1984, 25, 2307-2310; Lopez *et al. Tetrahedron: Asymmetry* 1991, 2, 93-96; each of which is incorporated herein by reference) in the context of projected Diels-Alder reactions of model ynolide **17** with diene **21**. Unfortunately, no product formation was observed.

We next directed our attentions to the use of the dimedonederived cyclic diene **25** (Ibuka, T.; Mori, Y.; Aoyama, T.; Inubushi, Y. *Chem. Pharm. Bull.* 1978, 26, 456-465; Langer, P.; Schneider, T.; Stoll, M. *Chem. Eur. J.* 2000, 6, 3204-3214; each of which is incorporated herein by reference) (Scheme 3-9). The latter could, in principle, react with ynolide diastereomers **12** to afford desired product **22** after elimination of isobutylene (Uchiyama *et al. Tetrahedron Len.* 2000, 41, 10013-10017; Morrison *et al. Tetrahedron Len.* 2001, 42, 7367-7369; incorporated herein by reference). The possible advantages of this diene over tri-oxygen-substituted acyclic possibilities, such as **20** or **21**, are that the cyclic diene could well be more reactive (due to a locking in of the *s*-syn conformation by the six-member ring) and more thermally stable. Happily, when diene **25** and dienophile **12** were heated to 160 °C, followed by desilylation, in the course of silica gel chromatography, the desired product, **22**, was obtained in 78% yield. Thus a new, highly efficient and convergent method to assemble the resorcinylic macrolide **22**, needed for cyclopropparadicicol, had been achieved.

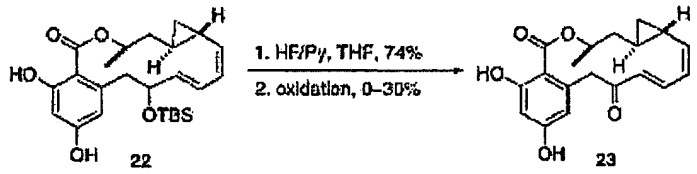


Scheme 3-9. Diels-Alder Reaction of **12** with Cyclic Diene **25**

Completion of the Synthesis. To complete the synthesis of cyclopropparadicicol (**2**), two remaining steps were required. Oxidation of the 2° alcohols to the corresponding ketone and regioselectve introduction of a chlorine had to be accomplished in some as yet unspecified order. We first attempted to carry out the

oxidation of the secondary homobenzylic alcohol in the presence of the free phenolic hydroxyls (Scheme 3-10). Following the removal of the TBS ether group (using HF/pyridine) in 74% yield, we surveyed a variety of oxidation conditions. Unfortunately, in the best case, we obtained only 30% yield through the use of activated MnO_2 (Gritter *et al. J. Org. Chem.* 1959, 24, 1051-1056; Trost *et al. J. Org. Chem.* 1983, 48, 3252-3265; each of which is incorporated herein by reference). The use of other oxidizing agents examined resulted in the decomposition of starting material, **22** (Huang *et al. J. Org. Chem.* 1976, 41, 3329-3231; Hauser *et al. J. Org. Chem.* 1983, 48, 1328-1333; Welch *et al. Synth. Commute* 1993, 23, 131-134; Tatsuta *et al. Chem. Len.* 2001, 172-173; Ley *et al. Synthesis* 1994, 639-666; each of which is incorporated herein by reference).

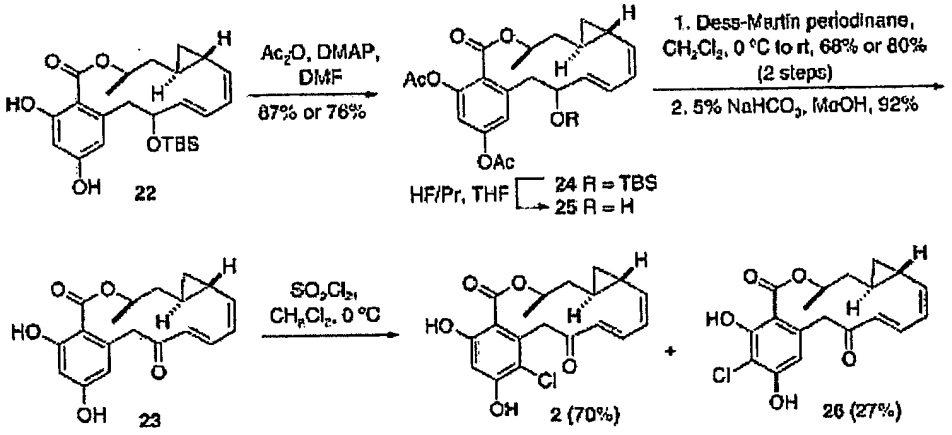
Scheme 3-10. Oxidation in the Presence of Free Phenols



Entry	Oxidation conditions	Yields
1	MnO_2	< 30%
2	TFAA/DMSO/Et_3N, CH_2Cl_2	Decomposition
3	PCC, NaOAc, CH_2Cl_2	Decomposition
4	Dess-Martin periodinane, CH_2Cl_2	decomposition
5	TPAP, CH_2Cl_2, MS 4Å	decomposition

Accordingly, we were obliged to protect the two free phenolic hydroxyl groups. Treatment of **22** with acetic anhydride in DMF and cat. amount of *N,N*-dimethylamino pyridine (DMAP) provided diacetate **24** (87% from the major isomer of **22** and 76% from the minor isomer of **22**) (Scheme 11). Subsequent removal of its TBS ether gave alcohol **25**. This deprotection was followed by oxidation using Dess-Martin periodinane, to afford the desired ketone intermediate in 68% yield from the major isomer of **25** (80% yield from the minor isomer). The stereochemistry of two isomers

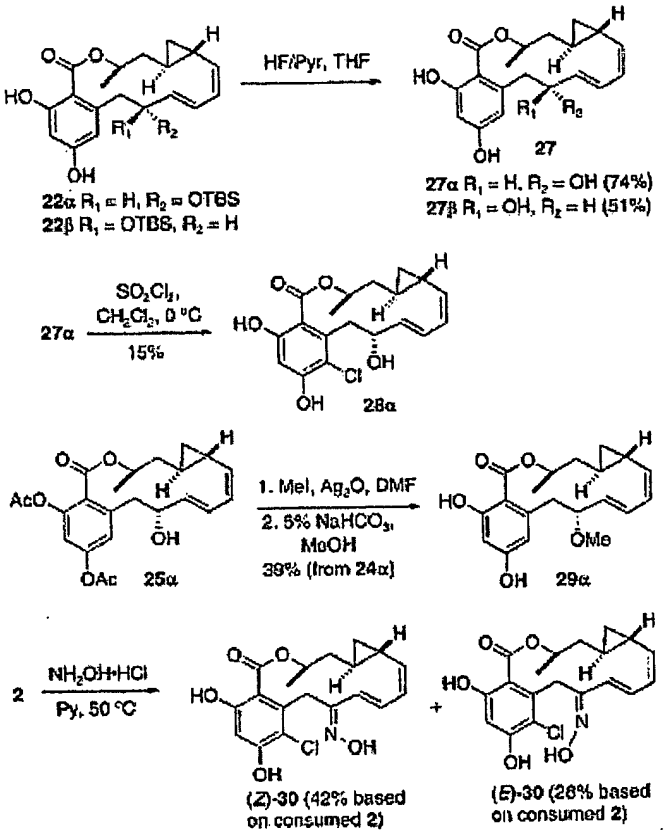
of **25** could be determined by a modified Mosher's analyses (see Experimentals for details) (Ohtani *et al. J. Org. Chem.* 1991, 56, 1296-1298; each of which is incorporated herein by reference). The acetate groups were cleaved under mildly basic conditions (5% $NaHCO_3$/MeOH, 1:1) in excellent yield (Garbaccio *et al. J. Am. Chem. Soc.* 2001, 123, 10903-10908; incorporated herein by reference). Finally, regioselective chlorination provided cycloproparadicicol (**2**) in 70% yield. Unfortunately, this product was accompanied by the formation of an isomeric chlorination product **26** in 27% yield (Garbaccio *et al. J. Am. Chem. Soc.* 2001, 123, 10903-10908; incorporated herein by reference). Although the issues governing such chlorinations have not necessarily been optimized, this level of regioselectivity seems to be quite general for such resorcinylic compounds (Elix *et al. Aust. J. Chem.* 1997, 50, 971-975; Elix *et al. Aust. J. Chem.* 1992, 45, 845-855; each of which is incorporated herein by reference). In summary, we have developed a new concise synthesis for cycloproparadicicol, in 6% yield following 13 steps.



Scheme 3-11. Completion of the Synthesis of **2**

Synthesis of Analogues and Formulation of a Preliminary SAR Pattern in the Cycloproparadicicol Series. The new synthesis developed here not only allowed us to generate sufficient quantities of cycloproparadicicol to support our biological studies on the compound itself but also allowed us to fashion a series of interesting analogues to study structure-activity relationships. To evaluate the biological impact of modification of the ketone group, two alcohol isomers **27**α,β were prepared (Scheme 3-12). Using SO_2Cl_2, the chloride derivative **28**α was synthesized in one step from the

major isomer, **27α**. The methyl ether derivative of **27α**, **29α** was also prepared under neutral conditions (MeI/Ag_2O) (Tanis *et al. J. Am. Chem. Soc.* 1992, 114, 8349-8362; incorporated herein by reference). Oxime analogues of cyclopropradicicol [(*Z*)- and (*E*)-**30**] were synthesized as well (Agatsuma *et al. Bioorg. Med. Chem.* 2002, 10, 3445-3454; incorporated herein by reference).

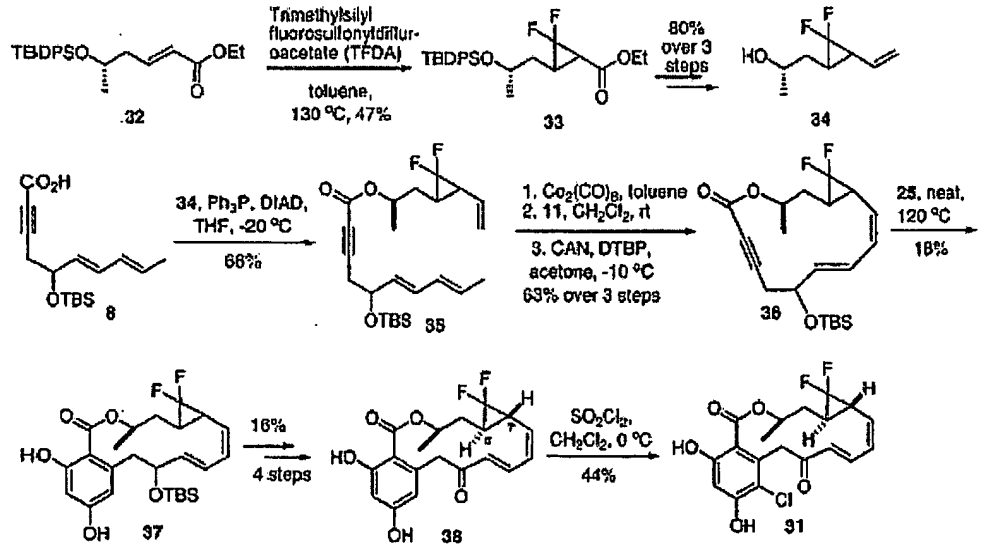


Scheme 3-12. Synthesis of Cyclopropradicicol Analogues

Synthesis of Difulorocyclopropradicicol. Our laboratory recently discovered that a trifluoro-derivative of epothilone exhibits a far superior in vivo profile (Chou *et al. Angew. Chem., Int. Ed 2003*, 42, 4762-4767; incorporated herein by reference). Accordingly, we sought to synthesize difluorocyclopropradicicol **31**, hoping to identify even better druglike molecules targeting Hsp90.

The difluorocyclopropane group was introduced to the known conjugated ester **32**, using reagents developed by Dolbier and co-workers, thereby affording difluoroesters **33** as a 1:1 mixture of stereoisomers (Scheme 3-13) (Tian *et al. Org.*

Lett. 2000, 2, 563-564; incorporated herein by reference). Our goal here was to achieve the target rapidly using the methodology already described in this account. Hence, the mixture of stereoisomers was carried forward through the required synthetic steps. As outlined in Scheme 3-13, the key ynolide **36** was prepared in reasonable yields following the now familiar protocols described above. The subsequent Diels-Alder reaction with diene **25**, however, proved to be very difficult, affording only 18% of product. Increasing the reaction temperature further eroded the yield, presumably due to the thermal instability of the difluorocyclopropane-containing ynolide **35**. Nevertheless, using the post-Diels-Alder sequence described previously, adequate amounts of **31** and **38** were obtained to support in vitro studies. It will be noted that, after Dess-Martin oxidation, ketone **38** was isolated as a single isomer. Seemingly, this difluoroyclopropyl isomer of **38** appears to have the same cyclopropane centers (7'R, 8'R) as does cycloproparadicicol itself, based on ^{1}H NMR and optical rotation comparisons (see the Experimentals below for details). Presumably, the alternate trans cyclopropyl diastereomer fares poorly in terms of yield in going through the steps from **35** to the end.

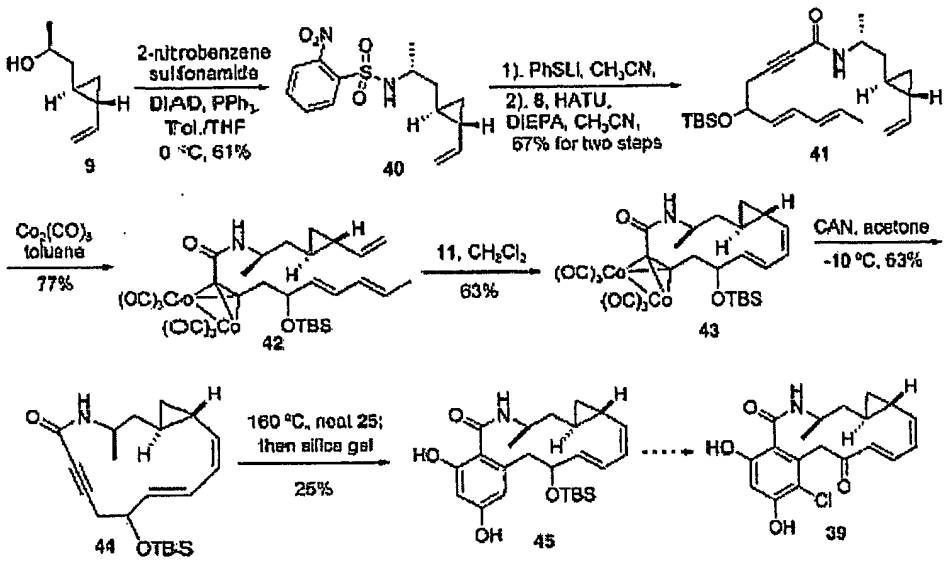


Scheme 3-13. Synthesis of Difluorocycloproparadicicol

Synthetic Approach to the Cycloproparadicicol Lactam. One of the structural features of radicicol that might have contributed to its instability in vivo, in

addition to the 7'-8' epoxide, is the macrolactone. The latter could be subject to metabolic hydrolysis by esterases. With this in mind, we also set out to synthesize the lactam version of the cycloproparadicicol (see compound **39**).

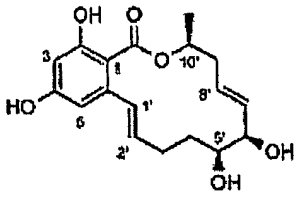
To introduce the functionality required to reach **39**, a sequence involving Staudinger reduction of an azide was considered (Stachel *et al. J. Org. Chem.* 2001, 66, 4369-4378; incorporated herein by reference). However, Mitsunobu type methodology, (Mitsunobu, O. *Synthesis* 1981, 1-28) including Thompson's protocol (Thompson *et al. J. Org. Chem.* 1993, 58, 5886-5888; incorporated herein by reference) failed to deliver the corresponding azide of compound **9**. This is probably due to the instability of the azide compound. Fortunately, it was found that the Nosyl approach recently developed by Fukuyama (Kurosawa *et al. J. Am. Chem. Soc.* 2003, 125, 8112-8113; incorporated herein by reference) afforded the desired sulfonamide **40** in 61% yield (Scheme 3-14). After the removal of the sulfonyl group, the resulting amine, generated in situ, coupled with alkynoic acid **8** to afford amide **41**. Application of the protocol described above (cobalt complexation → RCM →oxidative decomplexation) to the case at hand furnished "ynelactam" **44**. The Diels-Alder reaction of **44** with diene **25**, however, proved to be highly problematic. The best yield realized for reaching product **45** was only around 25%, and that proved to be difficultly reproducible. It is likely that replacement of the ester group by an amide function attenuates the electronegative pull on the acetylene, thereby eroding the reactivity of this linkage as a dienophile. Currently, investigations of other types of more reactive dienes are actively underway in our laboratory to deal with the problem of poorly reactive acetylenic dienophiles. Success on this front would allow us to operate more effectively with weakly reactive dienophiles such as **44**. Pending solution of the synthetic problem, studies directed to the synthesis of **39** have been deferred.



Scheme 3-14. Synthesis of Cyclopropparadicicol Lactam

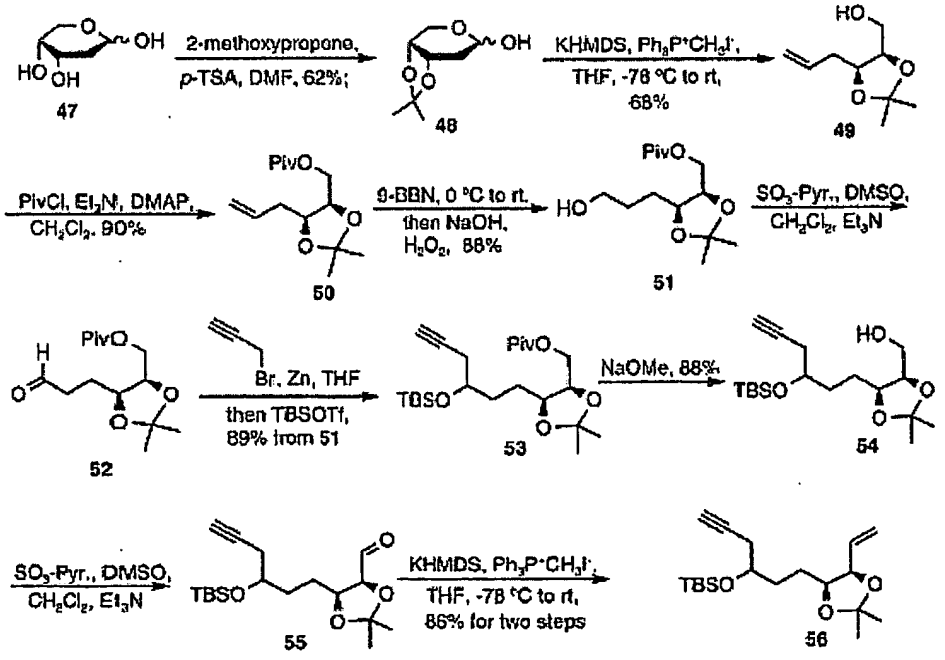
Extension of the Ynolide Approach to the First Total Synthesis of Aigialomycin D.

Nature has provided a variety of biologically significant natural products which share the same structural motif as radicicol, *i.e.*, a 14-member resorcinylic macrolide. We sought to test the scope and limitations of our newly developed ynolide-DA approach to such ring systems by applying it to an interesting member of this family, agialomycin D (**46**) (below). Recently, this compound was isolated from the marine mangrove fungus *Aigialus parvus* BCC5311 (Isaka *et al. J. Org. Chem.* 2002, 67, 1561-1566; incorporated herein by referened). It exhibited potent antimalarial activity (IC_{50}: 6.6 μg/mL against *P. falciparum*) and antitumor activity (IC_{50}: 3.0 μg/mL against KB cells) (Isaka *et al. J. Org. Chem.* 2002, 67, 1561-1566; incorporated herein by reference) We saw agialomycin D as an attractive candidate expanding upon our new "ynolide" strategy. An important issue to be overcome would be the proper emplacement of the E 1', 2' double bond requested to reach aigialomycin D. Also, the route must accommodate the hydroxy bearing stereogenic centers at carbons 5' and 6'.



Structure of Aigialomycin D

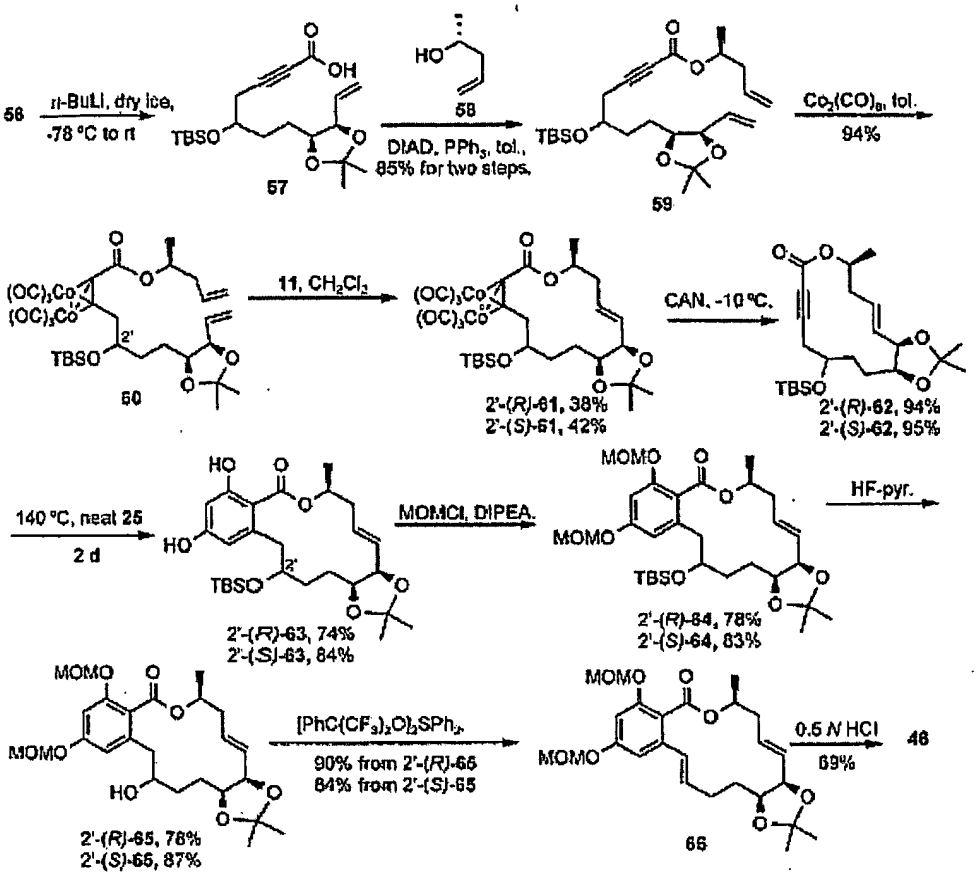
As shown in Scheme 3-15, the total synthesis commenced from the naturally derived D-2-deoxy-ribose **47**, which already has the desired future syn 5',6'-diol functionality of aigialomycin D in place. Compound **47** was processed forward via diol protection, Wittig olefination, hydroboration, and oxidation, to furnish key aldehyde **52**, as shown in Scheme 3-15. This compound was subjected to nucleophilic propargylation followed by protection of the resultant alcohol to afford TBS ether **53**. The pivaloyl group of **53** was then removed, and a vinyl group was installed through oxidation and Wittig reactions (see compound **56**).



Scheme 3-15. Synthesis of Alkyne **56**

Following chemistry of the type described above, compound **56** was advanced to ester **58** through carboxylation of the alkyne function and Mitsunobu esterification using the commercially available (*2R*)-4-penten-2-ol. We again investigated the possibility of closure on **58**, itself containing the free alkyne moiety as attempted above (see **10** and **14**). Again, starting material (**58**) was recovered following attempted RCM. Fortunately, we found that our newly discovered protocol (cobalt complexation followed by RCM and then decomplexation) proceeded very efficiently delivering ynolide **61**.

The two benzylic stereoisomers of **61** (2'-(*R*) and 2'-(*S*)) were separated by silica gel chromatography, and their absolute configurations were determined by modified Mosher's ester methodology (Ohtani *et al. J. Org. Chem.* 1991, 56, 1296-1298; incorporated herein by reference). As now expected, Diels-Alder reaction between **62** and diene **25** proceeded with high efficiency to afford the desired macrolide **63** in good yield. Following the protection of the phenol hydroxyl groups as the corresponding MOM ethers, the 2'-silyl group was removed and the dehydration reaction was carried out using the Martin sulfurane reagent (Martin *et al. J. Am. Chem. Soc.* 1971, 93, 4327-4329; incorporated herein by reference). Finally, global deprotection under acidic conditions completed the total synthesis of aigialomycin D **46**. The physical data (^{1}H and ^{13}C NMR, Mass spectrum, optical rotation, and IR) (Isaka *et al. J. Org. Chem.* 2002, 67, 1561-1566) in the context of the synthetic sequence and earlier spectral data serve to establish, independently, the structure of synthetic agialomycin D as shown. Moreover, the data for the final synthetic material are fully consistent with those reported for the natural product (Isaka, M.; Suyamsestakom, C.; Tanticharoen, M.; Kongsaeree, P.; Thebtaranonth, Y. *J. Org. Chem.* 2002, 67, 1561-1566; incorporated herein by reference).



Scheme 3-16. Completion of the Synthesis of Aigialomycin D

The accomplishment of the total synthesis of aigialomycin D demonstrates the generalizability of our newly developed protocol.

Biological Evaluation of Cycloproparadicicol Analogues. As discussed earlier, it appears that the inhibition of Hsp90 induces the proteasomal degradation of various oncogenic proteins, thereby leading to inhibition of tumor cell growth. We hoped to evaluate our synthetic analogues for their growth inhibition activity against MCF breast cancer cells. The IC_{50} values of our synthetic compounds were determined after treatment of the cells with each drug listed in Table 3-1 for 72 h. Cycloproparadicicol is the most potent of the new compounds. Interestingly, chloride regioisomer **26** was found to be inactive. Of the two alcohol isomers, **27**α displayed an IC_{50} value of 150 nM, and 27*β* is inactive. Further modifications of **27**α, however, decreased its potency. Surprisingly, introduction of the chlorine function (**28**α) resulted in raising the IC_{50} to 390 nM. Compound **29**a, methyl ether derivative of **27**α, is

completely inactive. The (*Z*)-oxime, (*Z*)-**30**, was found 3 times more potent that the corresponding (*E*)-isomer. Aigialomycin D was significantly less active than the cycloproparadicicol derivatives.

Table 3-1. IC50 (pM) Values of Cycloproparadicicol Analogues and Aigialomycin D

compound	2	26	27α	27β	28α	29α	(*Z*)-30	(*E*)-30	31	38	46
IC50	54	>500	150	>500	390	>10,000	98	282	10 000	3000	>10 000

To confirm that the cytotoxicity was indeed due to the inhibition of Hsp90, we set out to investigate the degradation of Her2, one of the most sensitive client proteins of this chaperone. The expression levels of Her2 of drug-treated MCF-7 cells were analyzed using immunoblotting (Figure 31). P85, used as a control, is not a client protein of Hsp90. As expected, the growth inhibition activities of the synthetic active analogues are reflected in their capacity to degrade Her2. The three most active compounds, **2**, **27α**, and (*Z*)-**30**, all were able to degrade Her2 at 0.3 μM. (*E*)-**30** is less effective. Nonchlorinated difluoro compound **38** degraded Her2 at 1 μM, and its chlorinated counterpart **31**, at 10 μM. Aigialomycin D (**46**) did not degrade the protein even at 10 μM.

Conclusions and Future Directions

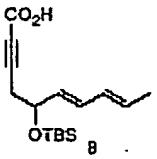
In summary, we have developed a new and efficient synthesis enabling the full evaluation of cycloproparadicicol as a feasible candidate for further advancement. The highlights of the new synthesis are cobalt-complexation-promoted RCM to generate ynolides, followed by Diels-Alder reaction with dimedonederived bis-siloxyl diene to assemble the benzofused macrolides. Given this pathway, we can generate grain quantities of cyloproparadicicol for biological evaluations. The generality of the synthesis plan has been demonstrated by its application to the first total synthesis of aigialomycin D.

It is, however, appropriate to point out that the sluggish dienophilicity of monoactivated acetylenes is still a problem preventing full generalizability of the method. For instance, in the work described above, *i.e.*, in the cases of the yne lactam **44** and the ynolide **36** containing the sensitive difluorcyclopropyl group, the yields of the Diels-Alder step are low.

The biological activities of our synthetic analogues shown here, in conjunction with the earlier demonstration that cycloproparadicicol binds to Hsp90 at ca. 160 nM (Yamamoto *et al. Angew. Chem., Int. Ed.* 2003, 42, 1280-1284; incorporated herein by reference) support the notion of the latter as the likely target for this new group of anticancer agents. *In vivo* evaluations of cycloproparadicicol are in progress in various settings and will be described upon completion of full preclinical studies.

Experimentals

General Methods: Reagents obtained from commercial suppliers were used without further purification unless otherwise noted. THF, toluene, and methylene chloride was obtained from a dry solvent system (passed through a prepacked column of alumina) and used without further drying. All air and water sensitive reactions were performed in oven or flame-dried glassware. NMR (^{1}H and ^{13}C) spectra were recorded on Bruker AMX-400 MHz or Bruker Advance DRX-500 MHz as noted individually, referenced to $CDCl_3$ (7.27 ppm for ^{1}H and 77.23 ppm for ^{13}C). Optical rotations were obtained on a JASCO model DIP-370 digital polarimeter. Low resolution mass spectra (ESI) were determined with a PESciex AP 130 spectrometer. High resolution mass spectra (FAB) were determined at Chemistry Department of Columbia University. Flash chromatography was performed with silica gel (230-400 mesh) from EM Science as the stationary phase. Analytical thin-layer chromatography was performed on E. Merck silica gel 60 F254 plates. Compounds which were not UV active were visualized by dipping the plates in phosphomolybdic acid solution and heating. Preparative thin layer chromatography was performed using the indicated solvent on Whatman® (LK6F Silica gel 60 Å 250 μM or Pk6F Silica Gel 60 Å 1000 μM) TLC plate.



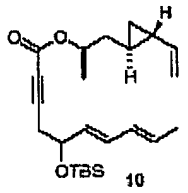
8

Acid **8**. To a suspension of activated zinc (15 g, 230 mmol) in dry THF (50 mL) at 0 °C was added propargyl bromide **5** (19.2 mL 80 wt% in toluene, 172 mmol). The resulting mixture was stirred at 0 °C for 1 hr, and sorbaldehyde **6** (12.7 mL, 115 mmol)

was added. After 1 hr at 0 °C, additional zinc (4.5 g, 69 mmol) was added, and stirring was continued for 2.5 hrs at room temperature (the reaction was exothermic and ice bath was needed occasionally to keep the temperature down). The reaction was quenched by slow addition of sat. aqueous NH_4Cl (500 mL), followed by diluting with Et_2O (1 L). The layers were separated, and the organic layer was washed with H_2O (300 mL), brine (300 mL), dried ($N_{a2}SO_4$), filtered and concentrated in vacuum. The residue was dissolved in CH_2Cl_2 (750 mL) with imidazole (9.8 g, 144 mmol), *t*-butyldimethylsilyl chloride (19 g, 126 mmol) and 4-(dimethylamino) pyridine (1.4 g, 11.5 mmol), and stirred at room temperature for 3 hrs. Additional imidazole (4.9 g, 72 mmol) and *t*-butyldimethylsilyl chloride (9.5 g, 63 mmol) were added, and stirring was continued for 9 hrs. The reaction was quenched by addition of sat. aqueous NH_4Cl (200 mL). The layers were separated, and the organic layer was washed with H_2O (200 mL), brine (200 mL), dried (Na_2SO_4), filtered and concentrated in vacuum. The residue was purified by flash chromatography (silica, 0 to 10% Et_2O in hexane) to give terminal alkyne precursor **7** (15 g, 52%). ^1H NMR ($CDCl_3$, 400 MHz) δ 6.18 (dd, J = 15.1, 10.5 Hz, 1H), 6.03 (ddd, J = 15.0, 10.6, 1.3 Hz, 1H), 5.72 (dd, J = 14.9, 6.9 Hz, 1H), 5.61 (dd, J = 15.1, 6.4 Hz, 1H), 4.30 (q, J = 6.3 Hz, 1H), 2.43 (ddd, J = 16.5, 6.2, 2.7 Hz, 1H), 2.34 (ddd, J = 16.5, 6.8, 1.7 Hz, 1H), 2.60 (t, J = 2.6 Hz, 1H), 1.77 (d, J = 6.8 Hz, 3H), 0.91 (s, 9H), 0.09, 0.06 (2s, 6H); ^{13}C NMR ($CDCl_3$, 100 Hz) δ 132.5, 131.1, 130.7, 130.0, 81.7, 72.1, 70.1, 28.9, 26.1, 18.4, -2.7; IR (film) ν_{max} 3313, 2956, 2930, 2856, 2121, 1255, 1115, 1079, 987, 837; ESIMS *m/z* 273 ([M + Na^+], $C_{15}H_{26}NaOSi$ requires 273).

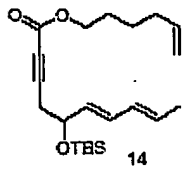
To a solution of **7** (15.0 g, 59.9 mmol) in Et_2O (270 mL) at -78 °C, was added a solution of BuLi (1.6 M in hexane, 41.5 mL, 66.4 mmol). After 45 min, excess crushed dry ice was added and the reaction was allowed to warm to room temperature. The solution was acidified by addition of 0.5 M aqueous citric acid (300 mL). The layers were separated, and the aqueous layer was extracted with additional Et_2O (300 mL x 2). The combined organic layers were dried ($MgSO_4$), filtered and concentrated in vacuum. The residue was purified by flash chromatography (silica, 50% to 100% EtOAc in hexane) to give product **8** as a light yellow solid (17.5 g, 99%). ^1H NMR ($CDCl_3$, 400 MHz) δ 6.19 (dd, J = 15.1, 10.4 Hz, 1H), 6.03 (ddd, J = 14.9, 10.6, 1.3 Hz, 1H), 5.75 (dd, J = 15.0, 6.8 Hz, 1H), 5.55 (dd, J = 15.1, 6.4 Hz, 1H), 4.36 (q, J = 6.3 Hz, 1H),

2.61-2.48 (m, 2H), 1.77 (d, J = 6.8 Hz, 3H), 0.91 (s, 9H), 0.10, 0.06 (2s, 6H); ^{13}C NMR ($CDCl_3$, 100 Hz) δ 157.6, 131.6, 131.3, 130.7, 89.7, 74.0, 71.4, 29.3, 26.0, 18.4, 18.3, -4.3, -4.7; IR (film) ν_{max} 2956, 2930, 2857, 2242, 1689, 1281, 1257, 1080; ESIMS *m/z* 317 ([M + Na^+], $C_{16}H_{26}NaO_3Si$ requires 317).



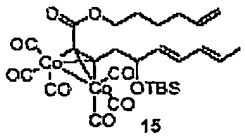
10

Alkynoic ester **10**. To a solution of DIAD (14.7 mL, 72.9 mmol) in dry THF (350 mL) was added Ph_3P (15.8 g, 60.2 mmol), and the mixture was stirred at room temperature for one hour. At -20 °C, a solution of acid **8** (13.1 g, 44.4 mmol) in 100 mL THF was added. After 15 min, a solution of alcohol **9** (4.0 g, 31.7 mmol) in 150 mL THF was added, and stirring was continued for 2 hours at -20 °C. The reaction was quenched by addition of 250 mL of pH 7.2 phosphate buffer, followed by warming to room temperature and diluting with EtOAc (1.5 L). The layers were separated, and the aqueous layer was extracted with EtOAc (2 x 250 mL). The combined organic layers were washed with brine (250 mL), dried (Na_2SO_4), filtered and concentrated in vacuum. The residue was purified by flash chromatography (silica, 50:1→20:1 hexanes/EtOAc) to give ester **10** as a mixture of two inseparable diastereoisomers (5.9 g, 47%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.17 (dd, J = 15.1, 10.5 Hz, 1H), 6.03 (ddd, J = 12.3, 10.6, 1.4 Hz, 1H), 5.70 (dd, J = 14.8, 6.8 Hz, 1H), 5.55 (dd, J = 15.2, 6.4 Hz, 1H), 5.37 (ddd, J = 17.1, 10.2, 8.7 Hz, 1H), 5.06 (q, J = 6.4 Hz, 1H), 5.03 (dd, J = 17.0, 1.5 Hz, 1H), 4.84 (dd, J = 10.2, 1.6 Hz, 1H), 4.34 (q, J = 6.4 Hz, 1H), 2.56-2.42 (m, 2H), 1.76 (d, J = 6.8 Hz, 3H), 1.57-1.53 (m, 2H), 1.29 (d, J = 6.4 Hz, 3H), 1.20-1.10 (m, 1H), 0.90 (s, 9H), 0.79-0.68 (m, 1H), 0.65-0.57 (m, 2H), 0.10, 0.05 (2s, 6H); ^{13}C NMR ($CDCl_3$, 100 Hz) δ 154.5, 141.4, 131.8, 131.1, 130.8, 130.4, 112.1, 86.2, 74.9, 73.0, 71.5, 39.7, 29.2, 26.0, 22.4, 19.8, 18.4, 18.3, 17.2, 13.7, -4.3, -4.7; IR (film) ν_{max} 2955, 2930, 2856, 2238, 1710, 1253, 1068; ESIMS *m/z* 437 ([M + Cl^-], $C_{24}H_{38}ClO_3Si$ requires 437); HRMS (FAB^+) *m/z* 403.2687 ([M + H]$^+$, $C_{24}H_{39}O_3Si$ requires 403.2668).



14

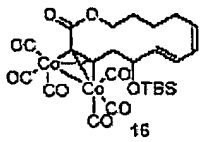
To a solution of acid **8** (192 mg, 0.653 mmol) and 5-hexen-1-ol (0.118 mL, 0.979 mmol) in dry CH_2Cl_2 (3 mL) was added EDCI (150 mg, 0.784 mmol) and 4-(dimethylamino)pyridine (8.0 mg, 0.065 mmol). After 3 hrs at room temperature, the reaction mixture was loaded on PTLC plates and purified (12:1 hexane/EtOAc) to give model ester **14** (146 mg, 59%).0 ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.18 (dd, J = 15.1, 10.5 Hz, 1H), 6.04 (ddd, J = 15.0, 10.5, 1.5 Hz, 1H), 5.79 (ddt, J = 17.1, 10.3, 7.2 Hz, 1H), 5.71 (dq, J = 15.0, 6.8 Hz, 1H), 5.56 (dd, J = 15.1, 6.4 Hz, 1H), 5.03 (dq, J = 17.1, 1.6 Hz, 1H), 4.97 (dd, J = 10.2, 1.6 Hz, 1H), 4.35 (q, J = 6.3 Hz, 1H), 4.16 (t, J = 6.6 Hz, 2H), 2.57-2.44 (m, 2H), 2.09 (q, J = 7.2 Hz, 1H), 1.77 (d, J = 6.9 Hz, 3H), 1.74-1.63 (m, 2H), 1.52-1.44 (m, 2H), 0.90 (s, 9H), 0.10, 0.06 (2s, 6H); ^{13}C NMR ($CDCl_3$, 100 Hz) δ 154.0, 138.4, 131.8, 131.1, 130.8, 130.5, 115.1, 86.6, 74.6, 71.5, 65.9, 33.4, 29.3, 28.1, 26.0, 25.3, 18.4, 18.3, -4.3, -4.7; IR (film) ν_{max} 2955, 2930, 2856, 2238, 1713, 1249, 1072; ESIMS *m/z* 399 ([M + Na^+], $C_{22}H_{36}NaO_3Si$ requires 399). HRMS (FAB^+) *m/z* 375.2363 ([M - H]$^+$, $C_{22}H_{35}O_3Si$ requires 375.2355).



15

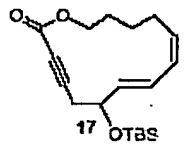
Cobalt complex **15**. To a solution of ester **14** (77.8 mg, 0.207 mmol) in toluene (9 mL) was added $Co_2(CO)_8$ (99.0 mg, 0.289 mmol). The mixture was stirred at room temperature for 45 min, and then concentrated in vacuum. The dark residue was purified by PTLC (15:1 hexane/EtOAc) to give cobalt complex **15** (117.5 mg, 86%) as a red oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.17 (dd, J = 15.3, 10.6 Hz, 1H), 6.03 (ddd, J = 15.3, 11.3 Hz, 1H), 5.68 (dd, J = 14.9, 6.9 Hz, 1H), 5.61 (dd, J = 15.2, 6.8 Hz, 1H), 5.38 (ddd, J = 17.1, 10.1, 8.7 Hz, 1H), 5.10 (q, J = 6.4 Hz, 1H), 5.04 (dd, J = 17.1, 1.3 Hz, 1H), 4.85 (dd, J = 10.3, 1.4 Hz, 1H), 4.41, (m, 1H), 3.20-3.15 (m, 2H), 1.76 (d, J =

6.7 Hz, 3H), 1.59 (t, J = 6.6 Hz, 2H), 1.32 (d, J = 6.2 Hz, 3H), 1. 22-1.17 (m, 1H), 0.90 (s, 9H), 0.82-0.72 (m, 1H), 0.66-0.59 (m, 2H), 0.09, 0.08 (2s, 6H); ^{13}C NMR ($CDCl_3$, 100 Hz) δ 198.7, 169.2, 141.4, 134.3, 132.5, 132.4, 132.2, 131.0, 130.2, 128.8, 127.2, 127.1, 112.0, 93.0, 81.0, 73.7, 73.6, 73.1, 40.0, 26.1, 22.4, 19.9, 18.6, 18.4, 17.4, 13.9, 13.5, -4.2, -4.3, -4.6; IR (film) ν_{max} 2956, 2930, 2858, 2097, 2058, 2029, 1703, 1221, 1065; ESIMS *m/z* 685 ([M + Na^+], $C_{28}H_{36}Co_2NaO_9Si$ requires 685).



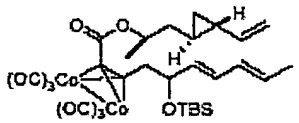
16

RCM product **16**. To a solution of cobalt complex **15** (16 mg, 0.024 mmol) in dry CH_2Cl_2 (120 mL) was added tricyclohexyl phosphine[1,3-bis(2,4,6-trimethylphenyl)-4,5-dihydroimidazol-2-ylidene]-[bezyli-dene] ruthenium(IV) dichloride (second generation Grubbs catalyst, **11**) (6.1 mg, 0.0072 mmol). The resulting solution was heated to 45 °C for 1 hr and 10 min, then cooled to room temperature and filtered through a plug of silica gel. The solvent was removed under reduced pressure. The residue was purified by PTLC (15:1 hexane/EtOAc) to give cyclic product **16** (10.5 mg, 71%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.44 (dd, J = 15.3, 10.8 Hz, 1H), 5.97 (t, J = 10.8 Hz, 1H), 5.58 (dd, J = 15.4, 7.5 Hz, 1H), 5.54 (dt, J = 10.1, 4.5 Hz, 1H), 4.57-4.47 (m, 2H), 4.25-4.20 (m, 1H), 3.45-3.36 (m, 2H), 2.45-2.37 (m, 1H), 2.13-1.05 (m, 1H), 1.99-1.79 (m, 1H), 1.80-1.62 (m, 2H), 1.51-1.41 (m, 1H), 0.92 (s, 9H), 0.11, 0.08 (2s, 6H); ^{13}C NMR ($CDCl_3$, 100 Hz) δ 199.9, 170.4, 135.2, 132.7, 129.0, 127.1, 92.8, 77.4, 73.1, 65.0, 45.2, 26.4, 26.1, 25.4, 18.5, 1.2, -4.2, -4.6; IR (film) ν_{max} 2955, 2930, 2857, 2098, 2059, 2027, 1702, 1213, 1057; ESIMS *m/z* 643 ([M + Na^+], $C_{25}H_{30}Co_2NaO_9Si$ requires 643).



17

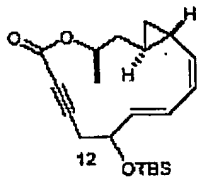
Model cyclic alkyne **11**. To a solution of compound **10** (35.6 mg, 0.0574 mmol) in acetone at -10 °C was added ammonium cerium (IV) nitrate (189 mg, 0.344 mmol) portionwise. After 10 min at -10 °C, the reaction was quenched by addition of diisopropylethylamine (0.18 mL, 1.03 mmol). The resulting mixture was filtered through a plug of neutral alumina, and the solvent was removed under reduced pressure. Purification by PTLC (15:1 hexane/EtOAc) afforded cyclic alkyne **11** (17.6 mg, 92%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.64 (dd, J = 15.5, 11.1 Hz, 1H), 6.07 (t, J = 11.0 Hz, 1H), 5.53 (dd, J = 15.5, 7.1 Hz, 1H), 5.40 (dt, J = 10.4, 4.8 Hz, 1H),), 4.41-4.25 (m, 2H), 4.06-4.01 (m, 1H), 2.69-2.62 (m, 1H), 2.56 (dd, J = 17.1, 4.4 Hz, 1H), 2.46 (dd, J = 17.1, 9.5 Hz, 1H), 2.26-2.21 (m, 1H), 1.75-1.61 (m, 4H), 0.89 (s, 9H), 0.08, 0.07 (2s, 6H); ^{13}C NMR ($CDCl_3$, 100 Hz) δ 153.9, 133.2, 132.9, 128.5, 128.2, 87.7, 77.0, 72.9, 68.0, 29.0, 28.2, 26.7, 26.0, 25.6, 18.3, -4.3, -4.7; IR (film) ν_{max} 2954, 2929, 2857, 2238, 1716, 1245, 1110, 1075, 837; ESIMS *m/z* 357 ([M + Na^+], $C_{19}H_{30}NaO_3Si$ requires 357). HRMS(FAB$^+$) *m/z* 333.1888 ([M - H]$^+$, $C_{19}H_{29}O_3Si$ requires 333.1886).



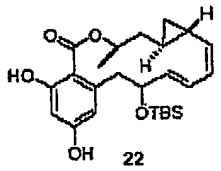
18

Cobalt complex **18**. To a solution of alkyne **10** (526 mg, 1.31 mmol) in toluene (60 mL) was added $Co_2(CO)_8$ (625 mg, 1.83 mmol). The mixture was stirred at room temperature for 30 min, and the solvent was removed under reduced pressure. The dark residue was purified by flash chromatography (silica, 0 to 5% EtOAc in hexane) to give cobalt complex **18** (902 mg, 100%) as an inseparable mixture of two diastereomers. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.17 (dd, J = 15.3, 10.6 Hz, 1H), 6.03 (ddd, J = 15.3, 11.3 Hz, 1H), 5.68 (dd, J = 14.9, 6.9 Hz, 1H), 5.61 (dd, J = 15.2, 6.8 Hz, 1H), 5.38 (ddd, J = 17.1, 10.1, 8.7 Hz, 1H), 5.10 (q, J = 6.4 Hz, 1H), 5.04 (dd, J = 17.1, 1.3 Hz, 1H), 4.85 (dd, J = 10.3, 1.4 Hz, 1H), 4.41, (m, 1H), 3.20-3.15 (m, 2H), 1.76 (d, J = 6.7 Hz, 3H), 1.59 (t, J = 6.6 Hz, 2H), 1.32 (d, J = 6.2 Hz, 3H), 1. 22-1.17 (m, 1H), 0.90 (s, 9H), 0.82-0.72 (m, 1H), 0.66-0.59 (m, 2H), 0.09, 0.08 (2s, 6H); ^{13}C NMR ($CDCl_3$, 100 Hz) δ 198.7, 169.2, 141.4, 134.3, 132.5, 132.4, 132.2, 131.0, 130.2, 128.8, 127.2, 127.1,

112.0, 93.0, 81.0, 73.7, 73.6, 73.1, 40.0, 26.1, 22.4, 19.9, 18.6, 18.4, 17.4, 13.9, 13.5, -4.2, -4.3, -4.6; IR (film) ν_{max} 2956, 2930, 2858, 2097, 2058, 2029, 1703, 1221, 1065; ESIMS *m/z* 711 ([M + Na^+], $C_{30}H_{38}Co_2NaO_9Si$ requires 711).

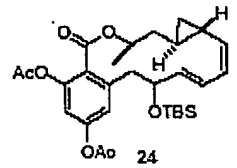


Cyclic alkyne **12**. To a solution of alkyne-cobalt complex **18** (575 mg, 0.84 mmol) in dry CH_2Cl_2 (4 L) was added tricyclohexyl phosphine[1,3-bis(2,4,6-trimethylphenyl)-4,5-dihydroimidazol-2-ylidene] [bezylidene] ruthenim(IV) dichloride (second generation Grubbs catalyst) (192 mg, 0.23 mmol). The resulting mixture was stirred at room temperature for 3.5 hours, and filtered through a short column of silica gel. The filtrate was concentrated in vacuum to give crude red oil product **19**. To a crude **19** (1.11 g, 0.0358 mmol) in acetone (100 mL) at -10 °C was added di*tert*butyl pyridine (5.8 mL, 25.8 mmol). Ceric ammonium nitrate (5.7 g, 10.3 mmol) was added in three portions. After 30 minutes at -10 °C, the reaction was quenched by the addition of a diisopropylethylamine (5.4 mL, 31 mmol), followed by warming to room temperature and diluting with CH_2Cl_2 (50 mL). The mixture was filtered through a pad of neutral alumna, and washed with generous amount of CH_2Cl_2. The filtrate was concentrated in vacuum. The residue was purified by PTLC (15:1, hexanes/EtOAc) to give cyclic alkyne **12** as two separable isomers (major 245.3 mg, 40%; minor 68.8 mg, 11%). Major isomer: ^1H NMR ($CDCl_3$, 400 MHz) δ 6.70 (dd, *J* = 15.6, 11.1 Hz, 1H), 5.98 (t, *J* = 10.8 Hz, 1H), 5.51 (dd, *J* = 15.6, 7.7 Hz, 1H), 5.20-5.14 (m, 1H), 4.92 (t, *J* = 10.7 Hz, 1H), 4.38-4.33 (m, 1H), 2.59 (dd, *J* = 16.8, 4.3 Hz, 1H), 2.41 (dd, *J* = 16.7, 10.9 Hz, 1H), 2.07 (dt, *J* = 14.8, 1.8 Hz, 1H), 1.74-1.67 (m, 1H), 1.33 (d, *J* = 6.5 Hz, 3H), 1.19-1.12 (m, 1H), 0.93-0.87 (m, 1H), 0.89 (s, 9H), 0.69-0.65 (m, 1H), 0.63-0.58 (m, 1H), 0.07 (s, 6H); ^{13}C NMR ($CDCl_3$, 100 Hz) δ 153.4, 136.5, 132.5, 128.3, 125.4, 85.7, 77.4, 73.8, 72.0, 38.6, 29.2, 26.0, 20.4, 18.4, 18.2, 14.6, 12.8; IR (film) ν_{max} 2954, 2928, 2856, 2238, 1706, 1252, 1105, 1072, 1004; ESIMS *m/z* 383 ([M + Na^+], $C_{21}H_{23}NaO_3Si$ requires a 383); HRMS (FAB^+) *m/z* 360.2130 ($[M]^+$, $C_{21}H_{32}O_3Si$ requires 360.2121). $[\alpha]^{25}_D$ +56 (*c* 0.95, $CHCl_3$).



22

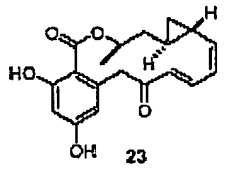
Diels-Alder product **22**. Compound **16** (major isomer, 1.8 g, 5.0 mmol) was dissolved in diene **25** (14 mL, 49 mmol) and heated to 160 °C in a sealed vial for 66 hours. The reaction mixture was cooled to room temperature and purified by silica gel chromatography (9:1 hexanes/EtOAc) followed by PTLC (4:1, hexanes/EtOAc) to give **22** (2.2 g, 99%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 11.58 (s, 1H), 6.56 (dd, J = 15.9, 9.3 Hz, 1H), 6.38 (d, J = 2.4 Hz, 1H), 6.33 (d, J = 2.4 Hz, 1H), 5.94 (t, J = 9.5 Hz, 1H), 5.68 (dd, J = 15.9, 6.8 Hz, 1H), 5.46-5.43 (m, 1H), 5.34 (dd, J = 10.1, 4.3 Hz, 1H), 4.51 (q, J = 6.7 Hz, 1H), 3.79 (dd, J = 13.6, 6.2 Hz, 1H), 2.81 (dd, J = 13.1, 8.9 Hz, 1H), 1.99-1.81 (m, 2H), 1.44 (d, J = 6.5 Hz, 3H), 1.21-1.13 (m, 1H), 0.93-0.87 (m, 1H), 0.88 (s, 9H), 0.58-0.51 (m, 2H), -0.02 (s, 6H); ^{13}C NMR ($CDCl_3$, 100 Hz) δ 164.8, 160.5, 143.8, 134.8, 133.7, 129.6, 128.0, 112.3, 106.1, 102.1, 75.7, 72.9, 42.6, 38.0, 26.1, 18.7, 18.4, 16.4, 16.1, 14.8, 1.4, -4.4, -4.6; IR (film) ν_{max} 3376, 2954, 2928, 2856, 1644, 1619, 1257, 1062, 835; ESIMS *m/z* 467 ([M + Na^+], $C_{25}H_{36}NaO_5Si$ requires 467). HRMS (FAB$^+$) *m/z* 444.2336 ([M]$^+$, $C_{25}H_{36}O_5Si$ requires 444.2332). $[\alpha]^{25}_D$ +3.1 (*c* 1.3, $CHCl_3$).



24

To a solution of **22** (major isomer, 243 mg, 0.546 mmol) in anhydrous DMF (13.5 mL) was added acetic anhydride (2.9 mL) and 4-(dimethylamino)pyridine (12.0 mg, 0.0546 mmol) sequentially. After 30 min at room temperature, the reaction was quenched by addition of 50 mL of pH 7.2 phosphate buffer. The resulting mixture was diluted with EtOAc (75 mL), separated and the aqueous layer was extracted with additional EtOAc (2 x 50 mL). The combined organic layers were washed with 5%

aqueous NaCl (2 x 45 mL). The combined washings were extracted with EtOAc (2 x 30 mL). The combined organic layers were washed with sat. aqueous NaCl (30 mL), dried (Na_2SO_4), filtered and concentrated. Purification of the residue by PTLC (4:1 hexane/EtOAc) gave diacetate **18** (250 mg, 87%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.96 (d, J = 2.1 Hz, 1H), 6.88 (d, J = 2.1 Hz, 1H), 6.59 (dd, J = 16.0, 10.5 Hz, 1H), 5.89 (t, J = 10.5 Hz, 1H), 5.66 (dd, J = 16.1, 4.9 Hz, 1H), 5.26-5.23 (m, 2H), 4.51 (q, J = 5.8 Hz, 1H), 3.12-3.02 (m, 2H), 2.28, 2.23 (2s, 6H), 1.47 (d, J = 6.2 Hz, 3H), 1.46-1.39 (m, 1H), 1.14 (ddd, J = 14.9, 10.1, 2.0 Hz, 1H), 0.96-0.87 (m, 1H), 0.87 (s, 9H), 0.52-0.48 (m, 2H), -0.01, -0.02 (2s, 6H); ^{13}C NMR ($CDCl_3$, 100 Hz) δ 168.6, 168.4, 166.4, 151.4, 148.7, 139.1, 135.9, 133.6, 129.2, 127.2, 125.6, 120.4, 114.7, 73.8, 73.2, 43.2, 40.0, 26.0, 21.3, 21.0, 19.3, 18.3, 16.9, 16.2, 17.7, -4.6, -4.7; IR (film) ν_{max} 2954, 2928, 2856, 1775, 1720, 1612, 1191, 1134, 1069; ESIMS m/z 551 ([M + Na^+], $C_{29}H_{40}NaO_7Si$ requires 551); HRMS (FAB$^+$) m/z 528.2569 ([M]$^+$, $C_{29}H_{40}O_7Si$ requires 528.2543). $[\alpha]^{25}_D$ -38 (c 1.1, $CHCl_3$).

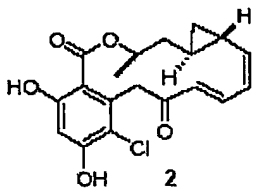


23

Cyclopropamonocillin **23**. To a solution of diacetate **24** (250 mg, 0.473 mmol) in THF (10.2 mL) at 0 °C was added pyridine (3.4 mL) and HF-Pyridine complex (1.7 mL) sequentially. The resulting mixture was stirred at room temperature for 10.5 hrs. TMSOMe (30 mL) was added, and stirring was continued for 45 min to quench the remaining HF. The solvents were removed under reduced pressure. The alcohol isolated was dried under high vacuum, and dissolved in CH_2Cl_2 (15 mL) and cooled to 0 °C. Dess-Martin periodinane (301 mg, 0.710 mmol) was added, and the resulting mixture was stirred at room temperature for 15 min. The solution was then directly loaded on PTLC plates and purified (1:1 hexane/EtOAc) to give the desired ketone (133 mg, 68%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.01 (dd, J = 16.1, 11.3 Hz, 1H), 6.97 (d, J = 1.4 Hz, 1H), 6.92 (d, J = 2.0 Hz, 1H), 6.20 (t, J = 10.4 Hz, 1H), 5.96 (d, J = 16.0 Hz, 1H), 5.60 (dd, J = 10.0, 7.2 Hz, 1H), 5.47-5.41 (m, 1H), 4.20 (d, J = 13.8 Hz, 1H),

3.77 (d, J = 13.8 Hz, 1H), 2.31 (dt, J = 15.3, 4.4 Hz, 1H), 2.25, 2.24 (2s, 6H), 1.73-1.71 (m, 1H), 1.50 (d, J = 6.5 Hz, 3H), 1.26-1.21 (m, 1H), 1.00-0.97 (m, 1H), 0.75-0.69 (m, 2H); ^{13}C NMR ($CDCl_3$, 125 Hz) δ 198.4, 168.5, 165.0, 152.1, 149.2, 145.6, 143.7, 135.7, 129.6, 128.8, 124.6, 119.1, 115.6, 72.8, 43.0, 38.3, 21.2, 18.2, 16.7, 16.5, 15.6; IR (film) ν_{max} 2928, 1774, 1728, 1657, 1621, 1586, 1290, 1190, 1132, 1029; ESIMS *m/z* 435 ([M + Na^+], $C_{23}H_{24}NaO_7$ requires 435); HRMS (FAB$^+$) *m/z* 413.1616 ([M + H]$^+$, $C_{23}H_{25}O_7$ requires 413.1600). $[\alpha]^{25}_D$ -269 (*c* 1.43, $CHCl_3$).

The above ketone (169 mg, 0.409 mmol) was dissolved in 26 mL 1:1 MeOH and 5% aqueous $NaHCO_3$, and stirred at room temperature for 14 hrs to remove the phenolic acetates. The resulting solution was diluted with sat. aqueous NH_4Cl (80 mL) and EtOAc (100 mL) and separated. The aqueous layer was extracted with additional EtOAc (3 x 75 mL). The combined organic layers were dried (Na_2SO_4), filtered and concentrated in vacuum. Purification by PTLC (1:1 hexane/EtOAc) provided **23** (124 mg, 92%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 11.33 (s, 1H), 8.36 (dd, J = 16.1, 11.7 Hz, 1H), 6.48 (d, J = 1.6 Hz, 1H), 6.40 (d, J = 2.5 Hz, 1H), 6.20 (t, J = 11.3 Hz, 1H), 6.01 (d, J = 15.9 Hz, 1H), 5.72 (dd, J = 9.8, 6.2 Hz, 1H), 5.49-5.45 (m, 1H), 5.32 (d, J = 13.6 Hz, 1H), 3.51 (d, J = 13.7 Hz, 1H), 2.31 (dt, J = 15.8, 3.3 Hz, 1H), 1.61-1.55 (m, 1H), 1.55 (d, J = 6.7 Hz, 3H), 1.32-1.28 (m, 1H), 1.03-0.97 (m, 1H), 0.74-0.72 (m, 1H), 0.64-0.63 (m, 1H); ^{1}C NMR ($CDCl_3$, 125 Hz) δ 201.8, 170.1, 165.6, 161.6, 145.3, 139.2, 129.5, 128.5, 109.4, 104.5, 102.7, 73.5, 43.2, 27.6, 17.9, 17.2, 15.9, 13.9; IR (film) ν_{max} 3260, 1650, 1618, 1586, 1447, 1259, 1160, 1099, 996, 854; ESIMS *m/z* 351 ([M + Na^+], $C_{19}H_{20}NaO_5$ requires 351); HRMS (FAB$^+$) *m/z* 329.1382 ([M + H]$^+$, $C_{19}H_{21}O_5$ requires 329.1389). (+)-(*R, R, S*)-**19**: $[\alpha]^{25}_D$ -189 (*c* 0.77, CH_2Cl_2).



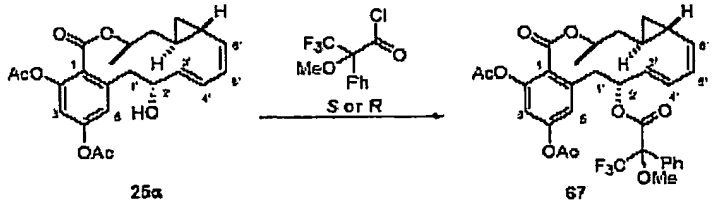
2

Cyclopropanaradicicol **2**. Compound **23** (90.4 mg, 0.275 mmol) was dissolved in dry CH_2Cl_2 (17 mL) and cooled to 0 °C. A solution of SO_2Cl_2 (0.035 mL, 0.44 mmol)) in CH_2Cl_2 (3.2 mL) was added dropwise. After 1 hour, the reaction was quenched by

the addition of 20 mL of 5% NH_4Cl, and diluted with CH_2Cl_2. The layers were separated, and the organic phase was washed with brine, dried ($MgSO_4$), filtered and concentrated in vacuum. Purification of the residue by PTLC (3:1, hexanes/EtOAc) gave **2** as a white solid (68.8 mg, 70%), along with regioisomer 26 (26.8 mg, 27%). ^1H NMR ($CDCl_3$, 500 MHz) δ 10.89 (s, 1H), 8.00 (dd, J = 15.9, 11.3 Hz, 1H), 6.63 (s, 1H), 6.42 (s, 1H), 6.13 (t, J = 11.2 Hz, 1H), 6.04 (d, J = 16.3 Hz, 1H), 5.62 (dd, J = 9.9, 5.8 Hz, 1H), 5.46-5.42 (m, 1H), 4.90 (broad d, J = 15.9 Hz, 1H), 3.77 (bs, 1H), 2.22 (dt, J = 15.8, 3.2 Hz, 1H), 1.60-1.52 (m, 1H), 1.48 (d, J = 6.7 Hz, 3H), 1.15-1.11 (m, 1H), 0.90-0.85 (m, 1H), 0.69-0.65 (m, 1H), 0.57-0.54 (m, 1H); ^{13}C NMR ($CDCl_3$, 125 Hz) δ 199.2, 169.4, 162.7, 155.6, 143.5, 142.5, 136.9, 129.9, 128.9, 115.6, 107.8, 103.5, 74.4, 46.4, 37.4, 17.9, 17.3, 15.6, 13.6; IR (film) ν_{max} 3341, 1716, 1651, 1609, 1578 cm^{-1}; ESIMS *m/z* 385 ([M + Na^+], $C_{19}H_{19}NaO_5Cl$ requires 385); HRMS (ESI) *m/z* 385.0820 ([M + Na^+], $C_{19}H_{19}NaO_5Cl$ requires 385.0819). (+)-(*R, R, S*)-**2**: $[\alpha]^{25}_D$ +69 (*c* 0.87, CH_2Cl_2).

Regioisomer **26**: ^1H NMR (500 MHz, $CDCl_3$): δ 12.02 (s, 1H), 8.25 (dd, J = 15.9, 11.4 Hz, 1H), 7.56 (s, 1H), 6.63 (s, 1H), 6.20 (d, J = 11.3, 11.2 Hz, 1H), 6.02 (d, J = 16.0 Hz, 1H), 5.70 (dd, J = 9.8, 6.2 Hz, 1H), 5.52-5.48 (m, 1H), 5.26 (d, J = 13.8 Hz, 1H), 3.50 (d, J = 13.8 Hz, 1H), 2.33 (dt, J = 15.9, 3.2 Hz, 1H), 1.56 (d, J = 6.7Hz, 3H), 1.30-1.25 (m, 1H), 1.01-0.92 (m, 1H), 0.74-0.71 (m, 1H), 0.65-0.62 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 200.8, 170.3, 160.6, 157.0, 145.2, 144.9, 137.5, 129.7, 129.0, 117.8, 109.1, 74.6, 73.8, 43.4, 37.8, 18.1, 17.4, 15.8, 14.1; ESIMS *m/z* 384.9 ([M + Na^+], $C_{19}H_{19}ClNaO_5$ requires 385.1).

Determination of the stereochemistry at the **2'** position of **25α**:



25α 67

The stereochemistry of **2'** of **25α** was determined according to the protocol reported by Ikuko Ohtani *et al.* (Ohtani, I.; Kusumi, T.; Kashman, Y.; Kakisawa, H. *J. Org. Chem.* **1991**, *56*, 1296-1298; incorporated herein by reference)/

To a solution of **25α** (7 mg, 0.017 mmol) in CH_2Cl_2 (0.15 mL) was added triethylamine (0.033mL, 0.238 mmol), DMAP (0.5 mg, 0.004 mmol) and (*S*)-or (*R*)-methoxytrifluorophenyl acetic chloride (0.025 mL, 0.136 mmol). The reaction mixture was stirred overnight before loaded onto PTLC (250 μm) and eluted by Hex/EtOAc (4/1). The product (*R*) or (*S*) ester was isolated as colorless oil (7.5 mg, 70%).

$\Delta\delta = \delta_S - \delta_R$.

$\Delta\delta_{3'} = \delta_{S3'} - \delta_{R3'} = 5.55\text{-}5.68 = -0.13$ ppm;

$\Delta\delta_{4'} = \delta_{S4'} - \delta_{R4'} = 6.12\text{-}6.14 = -0.02$ ppm;

$\Delta\delta_{5'} = \delta_{S5'} - \delta_{R5'} = 5.85\text{-}5.91 = -0.06$ ppm;

$\Delta\delta_{6'} = \delta_{S6'} - \delta_{R6'} = 5.16\text{-}5.18 = -0.02$ ppm;

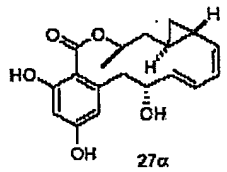
$\Delta\delta_{1'a} = \delta_{S1'a} - \delta_{R1'a} = 3.36\text{-}3.25 = 0.01$ ppm;

$\Delta\delta_{1'b} = \delta_{S1'b} - \delta_{R1'b} = 3.23\text{-}3.13 = 0.10$ ppm;

$\Delta\delta_5 = \delta_{S5} - \delta_{R5} = 6.93\text{-}6.92 = 0.01$ ppm;

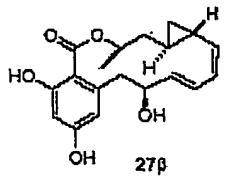
$\Delta\delta_3 = \delta_{S3} - \delta_{R3} = 7.16\text{-}7.14 = 0.02$ ppm.

Therefore, the 2' position should have a *R* configuration as shown above.



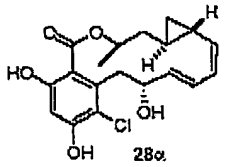
27α

Alcohol **27α** (major isomer). ^{1}H NMR (500 MHz, $CDCl_3$): δ 11.6 (s, 1H), 6.81 (dd, *J* = 15.8, 9.4 Hz, 1H), 6.39 (d, *J* = 2.4 Hz, 1H), 6.33 (d, *J* = 2.3 Hz, 1H), 5.94 (dd, *J* = 9.8, 9.7 Hz, 1H), 5.81 (dd, *J* = 15.8, 6.2 Hz, 1H), 5.42-5.38 (m, 1H), 5.33 (dd, *J* = 10.1, 5.0 Hz, 1H), 4.64 (m, 1H), 3.93 (dd, *J* = 13.8, 8.1 Hz, 1H), 2.93 (dd, *J* = 13.8, 5.9 Hz, 1H), 2.03 (dt, *J* = 15.7, 5.0 Hz, 1H), 1.88-1.80 (m, 1H), 1.49 (d, *J* = 6.6 Hz, 3H), 1.22-1.15 (m, 1H), 0.59-0.56 (m, 1H), 0.55-0.52 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 171.0, 165.3, 160.8, 143.2, 135.0, 132.6, 129.7, 129.4, 110.8, 106.1, 102.4, 74.4, 73.1, 40.5, 37.8, 18.7, 16.5, 16.1, 14.4; ESIMS *m/z* 353.0 ([M + Na^+], $C_{19}H_{22}NaO_5$ requires 353.1).



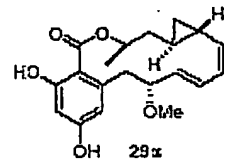
27β

Alcohol **27β** (minor). ^{1}H NMR (500 MHz, $CDCl_3$): δ 10.83 (s, 1H), 6.66 (dd, *J* = 16.3, 8.6 Hz, 1H), 6.52 (d, *J* = 2.6 Hz, 1H), 6.31 (d, *J* = 2.6 Hz, 1H), 5.89 (dd, *J* = 8.9, 7.6 Hz, 1H), 5.31-5.22 (m, 1H), 5.81 (dd, *J* = 15.8, 6.2 Hz, 1H), 5.26 (dd, *J* = 10.5, 5.9 Hz, 1H), 4.58-4.55 (m, 1H), 3.57-3.48 (m, 2H), 2.01-1.98 (m, 2H), 1.47 (d, *J* = 6.5 Hz, 3H), 0.97-0.92 (m, 1H), 0.64-0.57 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 169.8, 164.0, 160.2, 143.8, 134.7, 133.1, 128.1, 127.8, 111.3, 107.9, 102.4, 72.6, 72.4, 39.9, 37.6, 19.4, 16.7, 16.3, 14.7; ESIMS *m/z* 353.0 ([M + Na^+], $C_{19}H_{22}NaO_5$ requires 353.1).



28α

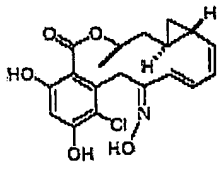
^{1}H NMR (500 MHz, $CDCl_3$): δ 11.4 (s, 1H), 7.14 (dd, *J* = 15.3, 11.1 Hz, 1H), 6.56 (s, 1H), 6.53-5.96 (m, 4H), 5.78 (dd, *J* = 15.6, 3.8 Hz), 5.38-5.33 (m, 1H), 4.55 (dd, *J* = 14.8, 6.7 Hz, 1H), 3.72 (dd, *J* = 14.6, 8.6 Hz, 1H), 3.57 (bs, 1H), 2.31-2.27 (m, 1H), 1.59-1.55 (m, 1H), 1.49 (d, *J* = 6.6 Hz, 3H), 0.91-0.88 (m, 1H), 0.58-0.54 (m, 1H), 0.43-0.38 (m, 1H); ESIMS *m/z* 387.0 ([M + Na^+], $C_{19}H_{21}NaClO_5$ requires 387.1).



29α

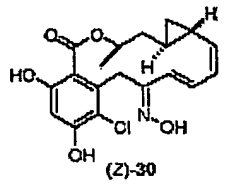
Methylether **29α**. To a solution of crude 25a (~ 0.033 mmol) in DMF (0.5 mL) was added CH_3I (0.0062 mL, 0.099 mmol), followed by the addition of Ag_2O (11.5 mg, 0.050 mmol). The mixture was stirred at rt for 21.5 h, and filtered through Celite. The solvent and then removed, and the resulting residue was dissolved in 2 mL of 1:1

mixture of MeOH and 5%$NaHCO_3$. After stirring at rt for 18.5 h, work-up and purification as described for **23** provided **29α** (4.4 mg, 39% over 3 steps). ^{1}H NMR (500 MHz, $CDCl_3$): δ 11.2 (s, 1H), 6.17 (dd, J = 15.9, 10.6 Hz, 1H), 6.24 (d, J = 1.6 Hz, 1H), 6.16 (d, J = 1.8 Hz, 1H), 5.77 (dd, J = 10.6, 10.5 Hz, 1H), 5.64 (dd, J = 15.9, 3.5 Hz, 1H), 5.15-5.10 (m, 1H), 4.99 (dd, J = 10.2, 6.6 Hz, 1H), 4.58 (m, 1H), 3.63 (s, 3H), 2.85 (dd, J = 14.8, 5.3 Hz, 1H), 2.73 (dd, J = 14.7, 9.5 Hz, 1H), 2.17 (dt, J = 15.2, 4.4 Hz, 1H), 1.36 (d, J = 6.3 Hz, 3H), 1.05-1.00 (m, 1H), 0.78-0.73 (m, 1H), 0.44-0.39 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 169.1, 158.9, 157.9, 137.1, 134.7, 134.2, 129.7, 127.6, 116.5, 106.5, 97.4, 73.0, 70.5, 55.8, 39.6, 39.2, 17.9, 16.2, 14.7; ESIMS *m/z* 367.2 ([M + Na^+], $C_{20}H_{24}NaO_5$ requires 367.2).



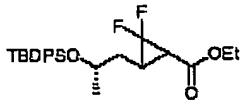
(*E*)-30

E-Oxime. Cycloproparadicicol **2** (4.6 mg, 0.0127 mmol) and hydroxylamine hydrochloride (2.2 mg, 0.032 mmol) were dissolved in pyridine (0.22 mL). The mixture was heated to 50 °C for 7 h. The pyridine was then removed in vacuum, and the residue was purified by PTLC (1:1, Hexanes/EtOAc) to afford two separable isomers along with recovered **2** (*E*-isomer: 1.0 mg, 28%; *Z*-isomer: 1.5 mg, 42% based on consumed starting material). ^{1}H NMR (400 MHz, $CDCl_3$) (*E*-isomer): δ 8.61 (d, J = 4.2 Hz, 1H), 7.46 (dd, J = 16.2, 11.2 Hz, 1H), 6.56 (s, 1H), 6.12 (d, J = 16.2 Hz, 1H), 6.04 (dd, J = 11.2, 9.8 Hz, 1H), 5.41-5.37 (m, 1H), 5.31 (dd, J = 9.8, 5.3 Hz, 1H), 4.62 (d, J = 16.3 Hz, 1H), 4.26 (d, J = 16.3 Hz, 1H), 2.17 (dt, J = 15.8, 2.8 Hz, 1H), 1.53-1.45 (m, 1H), 1.45 (d, J = 7.1 Hz, 3H), 1.03-0.97 (m, 1H), 0.79-0.71 (m, 1H), 0.58-0.53 (m, 1H), 0.45-0.42 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 169.4, 162.5, 157.3, 156.3, 138.3, 137.9, 135.6, 131.1, 124.4, 114.1, 108.9, 103.3, 74.3, 37.8, 28.3, 17.9, 17.3, 15.4, 13.0; ESIMS *m/z* 400.0 ([M + Na^+], $C_{19}H_{20}ClNNaO_5$ requires 400.1).



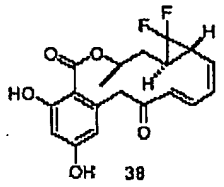
(Z)-30

Z-Oxime. ^{1}H NMR (400 MHz, $CDCl_3$) (Z-isomer): δ 8.60 (d, J = 4.3 Hz, 1H), 7.56 (dd, J = 15.5, 11.7 Hz, 1H), 6.75 (d, J = 16.3 Hz, 1H), 6.57 (s, 1H), 6.08 (dd, J = 10.8, 10.3 Hz, 1H), 5.43-5.39 (m, 2H), 4.78 (d, J = 14.7 Hz, 1H), 3.8 (m, 1H), 2.17 (d, J = 15.7, 1H), 1.55 (dd, J = 11.9, 11.5 Hz, 1H), 1.46 (d, J = 6.6 Hz, 3H), 0.87-0.80 (m, 2H), 0.61-0.56 (m, 1H), 0.49-0.46 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 169.3, 162.0, 156.5, 140.5, 137.3, 131.0, 117.3, 115.7, 108.7, 103.5, 74.2, 35.4, 18.2, 17.4, 15.6, 13.4; ESIMS *m/z* 400.2 ([M + Na^+], $C_{19}H_{20}ClNNaO_5$ requires 400.1).



33

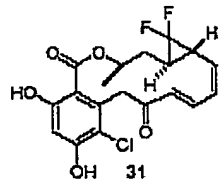
Difluorocyclopropanation products. To a solution of **32** (1.58 g, 3.98 mmol) in toluene (1 mL) was added NaF (25 mg, 0.58 mmol). Trimethylsilyl fluorosulfonyldifluroacetate (TFDA) (6.5 mL, 32.9 mmol) was added dropwise via a syringe pump at a rate of 1 mL/h. After 6.5 h, the reaction mixture was cooled to rt and filtered. The solvent was removed in vacuum. The residue was purified by PTLC (9:1 Hexanes/EtOAc) to give product as a mixture of diastereomers (830 mg, 47%, ratio 1/0.8). Major isomer: ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.75-7.72 (m, 5H), 7.49-7.27 (m, 5H), 4.26-4.19 (m, 2H), 4.06 (m, 1H), 2.55-2.49 (m, 1H), 2.13 (dd, J = 13.3, 7.5 Hz, 1H), 1.76-1.68 (m, 2H), 1.34-1.28 (m, 3H), 1.32 (d, J = 7.1 Hz, 3H), 1.15 (d, J = 6.6 Hz, 3H),1.12 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 166.9, 136.0, 134.5, 129.9, 127.9, 68.1, 61.5, 35.2, 34.9, 31.8, 31.4, 27.1, 26.1, 23.6, 19.3, 14.3. ESIMS *m/z* 469.1 ([M + Na^+], $C_{25}H_{32}F_2NaO_3Si$ requires 469.2).



38

Difluorocyclopropamonocilin. ^{1}H NMR (400 MHz, $CDCl_3$): δ 11.2 (s, 1H), 8.13 (dd, J = 16.0, 11.6 Hz, 1H), 7.50 (bs, 1H), 6.42 (d, J = 2.2 Hz, 1H), 6.40 (d, J = 2.2 Hz, 1H), 6.38 (t, J = 10.3 Hz, 1H), 6.02 (d, J = 16.0 Hz, 1H), 5.93 (dd, J = 10.0, 6.0 Hz, 1H), 5.59-5.56 (m, 1H), 5.24 (d, J = 13.7 Hz, 1H), 3.54 (d, J = 13.7 Hz, 1H), 2.18 (m, 1H), 1.01-1.99 (m, 1H), 1.65-1.55 (m, 2H), 1.49 (d, J = 6.9 Hz, 3H); $[\alpha]_D^{25}$ -17.1 (*c* 0.42, CH_2Cl_2); ESIMS *m/z* 386.9 ([M + Na^+], $C_{19}H_{18}F_2NaO_5$ requires 387.1).

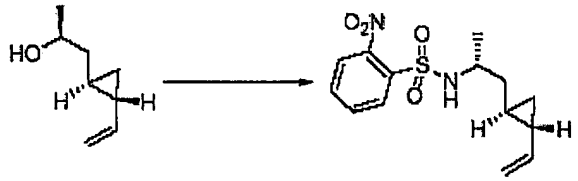
We assigned the stereo configuration at the difluorocyclopropyl group as same as that in cycloproparadicicol itself, based on the sign of known optical rotation of cycloproparadicicol ($[\alpha]_D^{25}$ -123.7 (*c* 0.70, CH_2Cl_2)) and its C7', 8'-epimer ($[\alpha]_D^{25}$ +221.3 (*c* 1.10, CH_2Cl_2) (Yamamoto, K.; Garbaccio, R. M.; Stachel, S. J.; Solit, D. B.; Chiosis, G.; Rosen, N.; Danishefsky, S. J. *Angew. Chem. Int. Ed.* **2003**, *42*, 1280-1284; incorporated herein by reference). Also, in the ^{1}H spectrum of **38**, H6' (5.93 ppm) has a coupling constant of 6.0 Hz with H7'. In the ^{1}H of cycloproparadicicol, a very similar coupling constant is observed between H6' and H7', *e.g.* 6.19 Hz (Yamamoto *et al. Angew. Chem. Int. Ed.* **2003**, *42*, 1280-1284; incorporated herein by reference). Of course, we realized that we will have to determine absolute stereochemistry of **38** by X-ray in the future when the material can be available after an improved synthesis is achieved.



31

Difluorocycloproparadicicol. ^{1}H NMR (400 MHz, $CDCl_3$): δ 11.2 (s, 1H), 7.69 (dd, J = 15.9, 10.7 Hz, 1H), 7.05 (bs, 1H), 6.38 (s, 1H), 6.38 (dd, J = 10.6, 9.7 Hz, 1H), 6.02 (d, J = 16.3 Hz, 1H), 5.93 (dd, J = 10.4, 5.6 Hz, 1H), 5.55 (m, 1H), 4.74 (d, J = 16.8 Hz, 1H), 3.92 (d, J = 17.0 Hz, 1H), 2.18 (m, 1H), 1.01-1.99 (m, 1H), 1.65-1.55 (m,

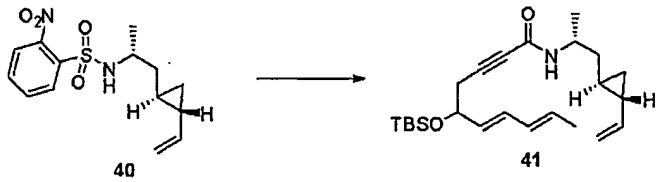
2H), 1.49 (d, J = 6.9 Hz, 3H); ESIMS *m/z* 433.0 ([M + Cl$^-$], $C_{19}H_{17}Cl_2F_2O_5$ requires 433.1).



9 40

Sulfonamide 40:

To a mixture of **9** (11 mg, 0.087 mmol), 2-nitrobenzenesulfonamide (44 mg, 0.218 mmol), triphenylphosphine (30 mg, 0.113 mmol) in toluene (0.4 mL) and THF (0.2 mL) was added diisopropylazadicarboxylate (DIAD, 0.022 mL, 0.113 mmol) at 0 °C. The reaction mixture was warmed up to r.t. and stirred for 2 hours. The reaction mixture was purifiedby PTLC (eluant: Hex/EtOAc = 3/1) to afford sulfonamide **40** as a colorless oil (17 mg, 61%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.11-0.40 (m, 1 H), 0.41-0.47 (m, 1 H), 0.57 (m, 1H), 1.03-1.10 (m, 1 H), 1.11 (d, J = 6.6 Hz, 1 H), 1.19-1.32 (m, 1 H), 1.43-1.47 (m, 1 H), 3.50-3.55 (m, 1 H), 4.69 (dd, J = 10.3, 1.4 Hz, 1 H), 4.88 (dd, J = 10.3, 7.0 Hz, 1 H), 5.11-5.18 (m, 1 H), 5.22 (d, J = 9.7 Hz, 1 H), 7.50-7.69 (m, 2 H), 7.75-7.78 (m, 1 H), 8.05-8.08 (m, 1 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 13.6, 17.6, 21.9, 26.5, 41.7, 51.7, 12.3, 125.7, 131.1, 133.2, 133.9, 141.4, 148.2, 171.6. LRMS (ESI) calcd for $C_{14}H_{18}N_2O_4SNa^+$ $[M+Na]^+$: 333.1, found 333.0. LRMS (ESI) calc'd for $C_{14}H_{18}N_2O_4SCl^-$ $[M+Cl]^-$: 345.1, found 345.0.

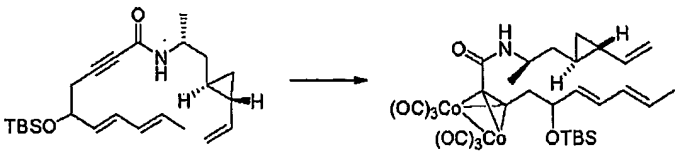


40 41

Amide 41:

To a solution of **40** (211 mg, 0.680 mmol) in CH_3CN (15 mL) was added PhSLi (1.0 M in THF, 1.1 mL, 1.02 mmol) and stirred for 1 h. Then a pre-mixed solution of

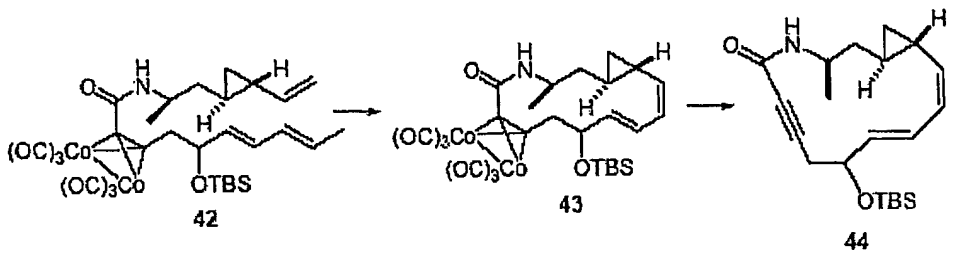
acid **8** (460 mg, 1.56 mmol), DIEA (0.28 mL, 1.63 mmol), HATU (647 mg, 1.7 mmol) in CH_3CN (4 mL) was added to the reaction mixture. After 0.5 h, the reaction mixture was concentrated and purified on a silica gel column (eluant: Hex/EtOAc = 4/1) to afford amid **41** as a colorless oil (182 mg, 67% for two steps). ^1H NMR (400 MHz, $CDCl_3$) δ 0.00 (s, 3 H), 0.03 (s, 3 H), 0.49-0.52 (m, 1 H), 0.57-0.60 (m, 1 H), 0.68-0.69 (m, 1 H), 0.85 (s, 9 H), 1.12 (d, J = 7.3 Hz, 1 H), 1.32-1.37 (m, 1 H), 1.41-1.46 (m, 1 H), 1.70 (d, J = 6.7 Hz, 1 H), 2.34-2.39 (dd, J = 16.7, 6.4 Hz, 2 H), 4.02-4.08 (m, 1 H), 4.24-4.30 (m, 1 H), 4.80 (d, J = 10.3 Hz, 1 H), 5.00 (d, J = 7.0 Hz, 1 H), 5.28-5.36 (m, 1 H), 5.48-5.53 (m, 2 H), 5.61-5.69 (m, 1 H), 5.69-5.61 (m, 1 H), 6.09-6.14 (m, 1 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ -5.9, -5.5, 0.0, 12.2, 16.2, 17.1, 17.2, 19.1, 21.7, 24.8, 27.8, 39.0, 45.0, 70.4, 82.7, 110.9, 127.4, 129.2, 129.6, 129.8, 130.7, 140.2, 151.5. HRMS (FAB) calc'd for $C_{24}H_{39}NO_2SiNa^+$ $[M+H]^+$: 424.2648, found: 424.2648, Δ = -0.1 ppm.



41 42

Cobalt complex 42:

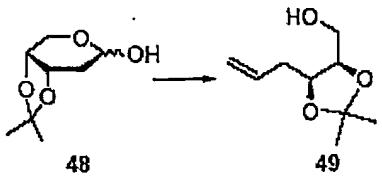
To a solution of **41** (669 mg, 1. 66 mmol) in toluene (50 mL) was added $Co_2(CO)_8$ (797 mg, 2.33 mmol). After 0.5 h, the reaction mixture was concentrated and purified on a silica gel column (eluant: Hex/EtOAc = 20/1) to afford Cobalt complex **42** as a purple oil (934 mg, 85%). ^1H NMR (400 MHz, $CDCl_3$) δ 0.00 (s, 3 H), 0.02 (s, 3 H), 0.48-0.50 (m, 1 H), 0.52-0.56 (m, 1 H), 0.66-0.68 (m, 1 H), 0.81 (s, 9 H), 1.07-1.10 (m, 1 H), 1.15 (d, J = 6.0 Hz, 1 H), 1.21 (m, 2 H), 1.65 (d, J = 6.7 Hz, 1 H), 3.21 (m, 2 H), 3.99-4.04 (m, 1 H), 4.33-4.40 (m, 1 H), 4.75 (d, J = 10.1 Hz, 1 H), 4.93 (d, J = 16.9 Hz, 1 H), 5.26-5.33 (m, 1 H), 5.51-5.65 (m, 2 H), 5.66 (m, 1 H), 5.90-5.96 (m, 1 H), 6.05-6.10 (m, 1 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ -4.8, -4.6, 13.5, 17.5, 18.1, 18.4, 20.4, 22.3, 25.9, 40.4, 43.0, 46.8, 73.2, 73.4, 85.1, 93.1, 111.8, 129.8, 130.9, 131.9, 132.2, 132.3, 166.6, 198.7. LRMS (ESI) calc'd for $C_{30}H_{39}Co_2NO_8SNa^+$ $[M+Na]^+$: 710.1, found 710.2.



Macrolactam 44:

To a solution of **42** (902 mg, 1.31 mmol) in CH_2Cl_2 (6.6 L) was added **11** (2nd generation Grubbs' catalyst, 446 mg, 0.52 mmol). The reaction mixture was stirred for 5 h and filtered through silical gel and washed with CH_2Cl_2 and then concentrated. To the residue was then added acetone (80 mL) and di-*tert*-butylpyridine (4.4 mL, 19.7 mmol) and cooled to 0 °C. To this mixture at 0 °C was added CAN (4.2 g, 7.87 mmol). After 0.5 h, DIEA (4.1 mL, 23.6 mmol) was added to quench the reaction and the mixture was filtered by neutral alumina and concentrated. The crude was purified by PTLC (eluant: Hex/EtOAc = 3/1) to afford two isomer of **44**. Major isomer of **44**: (176 mg, 37%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.00 (s, 3 H), 0.01 (s, 3 H), 0.60-0.61 (m, 1 H), 0.65-0.69 (m, 1 H), 0.73 (m, 1 H), 0.82 (s, 9 H), 0.85-0.98 (m, 1 H), 1.13 (d, J = 6.8 Hz, 1 H), 1.55-1.61 (m, 2 H), 1.97-2.01 (m, 1 H), 2.25 (dd, J = 16.4, 10.9 Hz, 1 H), 2.51 (dd, J = 16.4, 4.3, Hz, 1 H), 4.24-4.32 (m, 2 H), 4.79-4.84 (m, 1 H), 5.50 (dd, J = 15.4, 7.8 Hz, 1 H), 5.92-5.98 (m, 1 H), 6.56 (dd, J = 15.4, 11.2 Hz, 1 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ -4.5, -4.2, 1.0, 12.9, 15.1, 18.5, 19.8, 20.1, 26.2, 29.1, 38.3, 44.8, 74.0, 79.4, 84.1, 126.2, 127.0, 134.7, 135.4, 152.5. LRMS (ESI) calcd for $C_{21}H_{33}NO_2SiNa^+$ $[M+Na]^+$: 382.2, found 382.2.

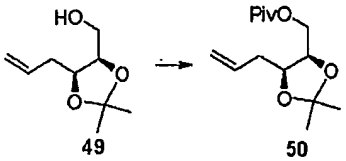
Minor isomer of **44** (108 mg, 23%). ^{1}H NMR (400 MHz, $CDCl_3$) δ -0.02 (s, 3 H), 0.00 (s, 3 H), 0.61-0.63 (m, 1 H), 0.64-0.70 (m, 2 H), 0.84 (s, 9 H), 0.92-0.99 (m, 1 H), 1.12 (d, J = 6.8 Hz, 1 H), 1.57-1.62 (m, 1 H), 1.97-2.02 (m, 1 H), 2.28 (dd, J = 16.9, 2.3 Hz, 1 H), 2.57 (dd, J = 16.9, 4.4, Hz, 1 H), 4.03-4.07 (m, 1 H), 4.48-4.49 (m, 1 H), 4.78 (t, J = 10.7 Hz, 1 H), 5.63 (dd, J = 15.0, 3.9 Hz, 1 H), 5.98 (t, J = 11.0 Hz, 1 H), 6.07 (bd, J = 9.0 Hz, 1 H), 6.95 (dd, J = 15.0, 11.4 Hz, 1 H). 13C NMR (100 MHz, $CDCl_3$) δ -6.1, -5.8, 0.0, 11.5, 13.7, 17.2, 18.3, 18.5, 24.7, 24.8, 27.3, 36.8, 43.4, 66.7, 77.4, 82.3, 125.3, 125.6, 131.7, 133.3, 151.5. HRMS (FAB) calcd for $C_{21}H_{33}NO_2SiNa^+$ $[M+Na]^+$: 382.2178, found 382.2178; Δ = -0.1 ppm.



48 49

(*2R*, *3S*)-Hex-5-ene-1,2,3-triol 2,3-acetonide (49):

To a stirring suspension of $Ph_3P^+CH_3I^+$ (11.2 g, 27.7 mmol) in 30 mL THF was added KHMDS (0.5 M in toluene, 46.0 mL, 23.0 mmol) at -78 °C. The solution was warmed up to 0 °C and stirred for 30 min before cooled down to -78 °C. Acetonide **48** (Barbat, J.; Gelas, J.; Horton, D. *Carbohydr. Res.* **1983**, *116*, 312-316; incorporated herein by reference) (1.6 g, 9.2 mmol) in 5 mL THF was added via cannula and the solution was warmed up to r.t. overnight (10 h) before quenched with saturated aqueous NH_4Cl solution, extracted with EtOAC (100 mL X 3). The organic layers were combined and dried with anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure vacuum. The residue was purified on a silica gel column using petroleum ether/EtOAc (4/1) as the eluant to afford **49** as a colorless oil (1.02 g, 68%). $[\alpha]_D^{25}$ 54.8 (*c* 0.26, $CHCl_3$). ^1H NMR (400 MHz,$CDCl_3$) δ 1.34 (s, 3 H), 1.46 (s, 3 H), 2.02 (b, 1 H), 2.25-2.32 (m, 1 H), 2.37-2.44 (m, 1 H), 3.65 (m, 1 H), 4.16-4.21 (m, 1 H), 4.24-4.28 (m, 1 H), 5.10-5.18 (m, 2 H), 5.79-5.89 (m, 1 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 25.4, 28.1, 33.6, 61.6, 76.2, 77.8, 108.3, 117.3, 134.2. LRMS (ESI) calcd for $C_9H_{16}O_3Na^+$ $[M+Na]^+$: 195.1, found 194.9. LRMS (ESI)calcd for $C_9H_{16}O_3Cl^-$ [M+Cl]: 207.1, found 207.1.

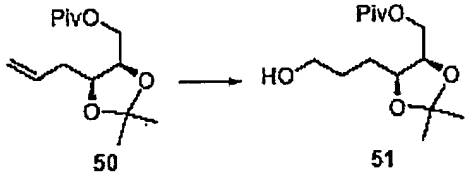


49 50

Pivalate (50):

To a solution of **49** (752 mg, 4.37 mmol), DMAP (106 mg, 0.874 mmol) and triethylamine (2.5 ml, 17.5 mmol) in CH_2Cl_2 (8 ml) was added PivCl (1.1 ml, 8.74 mmol) at 0 °C. The reaction mixture was warmed up to r.t. overnight (10h) before quenched with saturated aqueous $NaHCO_3$ solution, extracted with EtOAC (100 mL X

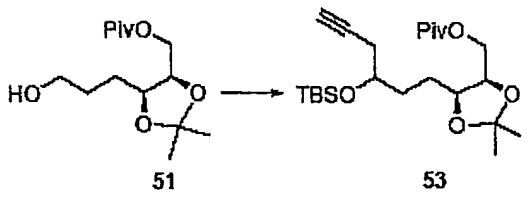
3). The organic layers were combined and dried with anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure vacuum. The residue was purified on a silica gel column using hexanes/EtOAc (4/1) as the eluant to afford **50** as a colorless oil (984 mg, 90%). $[\alpha]_D^{25}$ 15.6 (*c* 0.32, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) δ 1.22 (s, 9 H), 1.33 (s, 3 H), 1.47 (s, 3 H), 2.28-2.41 (m, 2 H), 4.10-4.13 (m, 2 H), 4.22-4.29 (m, 2 H), 5.10-5.17 (m, 2 H), 5.81-5.91 (m, 1 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 25.9, 27.5, 28.4, 39.1, 63.2, 75.5, 77.8, 108.8, 117.7, 134.6, 178.5. LRMS (ESI) calcd for $C_{14}H_{24}O_4Na^+$ $[M+Na]^+$ 279.2, found 278.9.



50 51

Alcohol **51**:

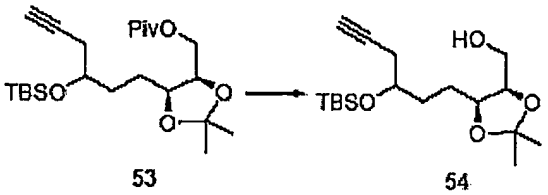
To a solution of **50** (166 mg, 0.648 mmol) in THF (1.5 mL) was added 9-BBN (0.5 M in THF, 2.8 mL, 1.425 mmol) at 0 °C. The reaction mixture was warmed up to r.t. over 4 h and H_2O (0.1 mL), NaOH (3 M, 0.7 mL) and H_2O_2 (30%, 0.2 mL) were added. The reaction mixture was diluted with H_2O 2.5 h later and acidified with citric acid (5%) till pH = 7. The mixture was extracted with EtOAc, washed with saturated aqueous $Na_2S_2O_3$, H_2O and brine. The organic layers were combined and dried with anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure vacuum. The residue was purified on a silica gel column using petroleum ether /EtOAc (1/1) as the eluant to afford **51** as a colorless oil (156 mg, 88%). $[\alpha]_D^{25}$ 82.4 (*c* 0.07, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) δ 1.21 (s, 9 H), 1.36 (s, 3 H), 1.46 (s, 3 H), 1.59-1.80 (m, 4 H), 2.30 (b, 1 H), 3.54 (m, 2 H), 4.08 (dd, *J* = 6.1, 11.5 Hz, 1 H), 4.12 (dd, *J* = 5.6, 11.5 Hz, 1 H), 4.19 (m, 1 H), 4.25 (m, 1 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 25.5, 25.9, 27.1, 28.0, 29.9, 38.7, 62.3, 62.9, 75.3, 77.0, 108.3, 178.2. HRMS (ESI) calcd for $C_{14}H_{26}O_5Na^+$ $[M+Na]^+$ 297.1678, found 297.1660, Δ = -6.0 ppm.



51 53

Alkyne 53:

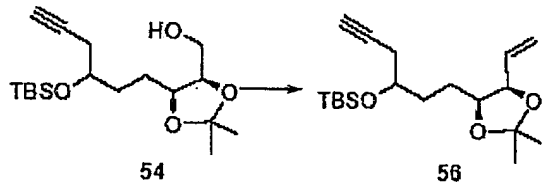
To a solution of **51** (682 mg, 2.485 mmol) in CH_2Cl_2 (5 mL), DMSO (5 mL) and Et_3N (3 mL) was added SO_3-pyridine complex (1.6 g, 10.2 mmol) at 0 °C. The reaction mixture was stirred for 1 h before diluted with EtOAc and washed with HCl (0.5 *N*), H_2O, saturated aqueous $NaHCO_3$ solution and brine. The organic layers were dried with anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure vacuum. The crude aldehyde **52** was used directly for next step without any further purification. To a suspension of Zn (Nano-size power, pre-activated, 390 mg, 5.949 mmol) in THF (10 mL) was added propargyl bromide (80% in toluene, 0.53 mL, 4.759 mmol) at 0 °C. The reaction mixture was stirred for 1 h at 0 °C and a solution of aldehyde **52** thus obtained in THF (5 mL) was added and reaction mixture was warmed up to r.t. over 2 h before quenched with saturated aqueous NH_4Cl solution and extracted with EtOAc. The organic layers were dried with anhydrous $MgSO_4$, filtered, and concentrated under vacuum. To a solution of crude alcohol thus obtained and 2,6-lutidine (0.6 mL, 4.76 mmol) in CH_2Cl_2 (8 mL) was added TBSOTf (0.82 mL, 3.57 mmol) and the reaction mixture was stirred for 10 h before quenched with saturated aqueous NH_4Cl solution, extracted with EtOAc (100 mL x 3). The organic layers were combined and dried with anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure vacuum. The residue was purified on a silica gel column using petroleum ether/EtOAc (20/1) as the eluant to afford **53** as a colorless oil (896 mg, 89% from **51**). ^1H NMR (400 MHz, $CDCl_3$) δ -0.02-0.05 (m, 6 H), 0.80 (s, 9 H), 1.13 (s, 9 H), 1.26 (s, 3 H), 1.36 (s, 3 H), 1.42-1.74 (m, 4 H), 1.81 (m, 1 H), 2.22-2.29 (m, 2 H), 3.78 (m, 1 H), 4.01-4.08 (m, 3 H), 4.12-4.17 (m, 1 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 18.0, 18.1, 24.3, 24.7, 25.6, 25.8, 27.0, 27.1, 27.2, 28.06, 28.07, 33.2, 33.3, 38.7, 62.8, 62.9, 70.1, 70.2, 70.4, 70.5, 75.2, 76.1, 77.1, 81.2, 108.20, 108.24, 178.1. HRMS (FAB) calcd for $C_{23}H_{42}O_5SiH^+$ $[M+H]^+$: 427.2880, found 427.2880, Δ = -0.1 ppm.



53 54

Alcohol 54:

To a solution of **53** (124 mg, 0.291 mmol) in MeOH (6 mL) was added NaOMe/MeOH (25%, 0.2 mL) and the reaction mixture was stirred for 10 h before quenched with saturated aqueous NH_4Cl solution, extracted with EtOAc (100 mL X 3). The organic layers were combined and dried with anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure vacuum. The residue was purified on a silica gel column using petroleum ether/EtOAc (8/1) as the eluant to afford **54** as a colorless oil (87 mg, 88%). ^1H NMR (400 MHz, $CDCl_3$) δ -0.02-0.00 (m, 6 H), 0.80 (s, 9 H), 1.32 (s, 3 H), 1.38 (s, 3 H), 1.32-1.91 (m, 4 H), 1.90 (m, 1 H), 1.96 (b, 1 H), 2.23-2.31 (m, 2 H), 3.53 (m, 2 H), 3.75 (m, 1 H), 4.08 (m, 1 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ -4.7, -4.6, -4.5, 18.0, 24.5, 24.7, 25.5, 25.6, 25.8, 27.2, 27.4, 28.2, 33.2, 33.4, 61.7, 70.1, 70.2, 70.46, 70.52, 76.9, 77.0, 77.9, 81.16, 81.24, 108.08, 108.14. HRMS (FAB) calcd for $C_{18}H_{34}O_4SiH^+$ $[M+H]^+$: 343.2305, found 343.2305, Δ = -0.1 ppm.

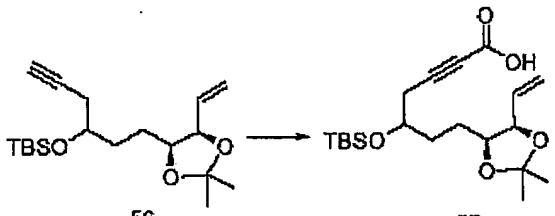


54 56

Enyne 56:

To a solution of **54** (87 mg, 0.254 mmol) in DMSO (1.0 mL), CH_2Cl_2 (1.0 mL) and Et_3N (1.0 mL) was added SO_3-Pyrdine complex (200 mg, 2.032 mmol) at 0 °C. The reaction mixture was stirred for 2 h before diluted with EtOAc and washed with HCl (0.5 *N*), H_2O, saturated aqueous $NaHCO_3$ solution and brine. The organic layers were dried with anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure vacuum. The crude aldehyde **55** was used directly for next step without any further purification. To a stirring suspension of $Ph_3P^+CH_3I^-$ (204 mg, 0.505 mmol) in 3 mL

THF was added KHMDS (0.5 M in toluene, 0.9 mL, 0.454 mmol) at -78 °C. The solution was warmed up to 0 °C and stirred for 30 min before cooled down to -78 °C. Aldehyde obtained as mention above in 2 mL THF was added via cannula and the solution was warmed up to r.t. overnight (10 h) before quenched with saturated aqueous NH_4Cl solution, extracted with EtOAc (100 mL X 3). The organic layers were combined and dried with anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure vacuum. The residue was purified on a silica gel column using petroleum ether/EtOAc (40/1) as the eluant to afford **56** as a colorless oil (73 mg, 86%). ^1H NMR (400 MHz, $CDCl_3$) δ -0.05 (s, 3 H), 0.02 (s, 3 H), 0.83 (s, 9 H), 1.27 (s, 3 H), 1.43 (s, 3 H), 1.43-1.78 (m, 4 H), 1.92 (b, 1 H), 2.23-2.31 (m, 2 H), 3.76 (m, 1 H), 4.05-4.10 (m, 1 H), 4.43-4.45 (m, 1 H), 5.02-5.05 (m, 2 H), 5.71-5.80 (m, 1 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ -4.30, -4.11, 18.4, 26.0, 26.1, 26.2, 26.5, 26.8, 27.7, 27.8, 28.62, 28.64, 33.2, 33.6, 70.4, 70.5, 71.07, 71.13, 78.6, 78.8, 80.2, 81.78, 81.82, 108.5, 118.6, 118.7, 134.7, 134.9. HRMS (FAB) calcd for $C_{19}H_{34}O_3SiNa^+$ $[M+Na]^+$: 361.2175, found 361.2175, Δ = 0.0 ppm.

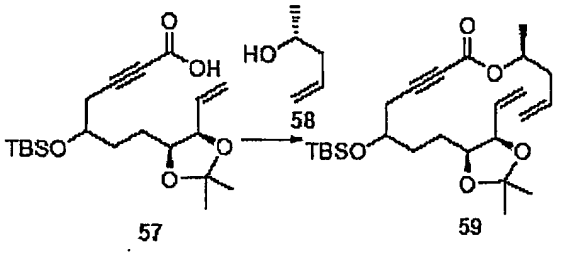


56 **57**

Acid 57:

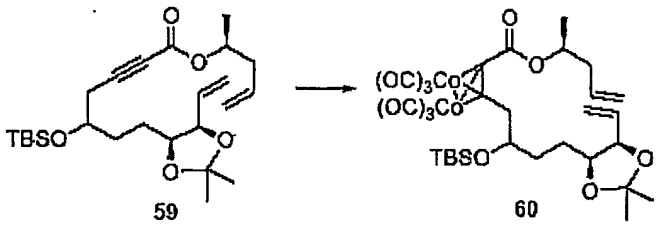
To a solution of **56** (586 mg, 1.731 mmol) in Et_2O (16 mL) was added BuLi (1.6 M in hexane, 1.817 mmol) at -78 °C and stirred for 5 min before quenched with dry ice and warmed up to r.t. The reaction mixture was washed with NaOH (0.1 M) and the aqueous layers were combined and acidified by HCl (0.1 M) until the pH = 2. The aqueous layer was extracted with EtOAc and the organic layers were combined and dried with anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure vacuum and dried on high vacuum. ^1H NMR (400 MHz, $CDCl_3$) δ -0.03 (s, 3 H), 0.00 (s, 3 H), 0.79 (s, 9 H), 1.17 (s, 3 H), 1.40 (s, 3 H), 0.95-1.68 (m, 4 H), 2.38-2.39 (m, 2 H), 3.80 (b, 1 H), 4.06 (m, 1 H), 4.43 (m, 1 H), 5.14-5.36 (m, 2 H), 5.67-5.76 (m, 1 H),

10.5 (bs, 1 H). This crude was used directly for next step without any further purification.



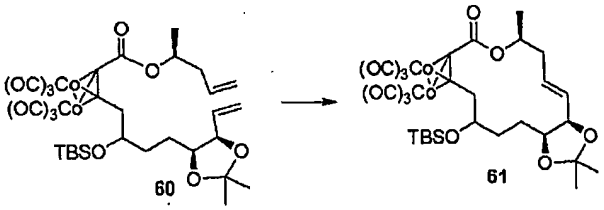
Ester 59:

To a solution of acid **57** (249 mg, 0.651 mmol) in toluene (15 mL) was added alcohol **58** (0.081 mL, 0.781 mmol), PPh_3 (205 mg, 0.781 mmol), DIAD (0.154 mL, 0.781 mmol). The reaction mixture was stirred for 10 h and the solvent was removed under reduced pressure vacuum. The residue was purified on a silica gel column using petroleum ether/EtOAc (40/1) as the eluant to afford **59** as a colorless oil (255 mg, 85% for two steps) . ^1H NMR (400 MHz, $CDCl_3$) δ -0.06-0.00 (m, 6 H), 0.80 (s, 9 H), 1.17 (d, J = 6.2 Hz, 3 H), 1.28 (s, 3 H), 1.38 (s, 3 H), 1.44-1.54 (m, 4 H), 2.20-2.24 (m, 1 H), 2.237-2.31 (m, 1 H), 2.35-2.37 (m, 2 H), 3.78-3.81 (m, 1 H), 4.03-4.05 (m, 1 H), 4.40-4.43 (m, 1 H), 4.94-4.96 (m, 1 H), 5.00-5.04 (m, 2 H), 5.14 (m, 1 H), 5.22 (dd, J = 6.7, 7.1 Hz, 1 H), 5.67-5.72 (m, 2 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ -4.64, -4.58, -4.55, 0.0, 18.0, 19.3, 25.6, 25.7, 25.8, 26.1, 26.3, 27.6, 27.7, 28.2, 28.3, 33.3, 33.6, 40.0, 70.10, 70.12, 72.0, 74.9, 78.1, 78.3, 79.80, 79.82, 86.1, 108.2, 108.3, 118.1, 118.3, 118.4, 133.2, 134.2, 134.4, 153.2. HRMS (FAB) calcd for $C_{25}H_{42}O_5SiH^+$ $[M+H]^+$: 451.2880, found 451.2881, Δ = -0.3 ppm.



Cobalt-complex 60:

To a solution of **59** (20 mg, 0.044 mmol) in toluene (2.5 mL) was added $Co_2(CO)_8$ (21.2 mg, 0.062 mmol). The reaction mixture was stirred for 30 min before filtered through neutral alumina and concentrated under reduced pressure vacuum. The residue was purified on preparative TLC (Whatman® Pk6F Silica Gel 60 Å 1000 μM) using Hexanes/EtOAc (20/1) as the eluant to afford **60** as a purple oil (30 mg, 94%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.07-0.09 (m, 6 H), 0.90 (s, 9 H), 1.30 (d, J = 8.1 Hz, 3 H), 1.33 (s, 1.6 H), 1.34 (s, 1.4 H), 1.45 (s, 1.6 H), 1.47 (s, 1.4 H), 1.25-1.46 (m, 1 H), 1.58-1.89 (m, 2.5 H), 1.89-1.95 (m, 0.5 H), 2.37-2.40 (m, 2 H), 3.00-3.12 (m, 2 H), 3.82-3.86 (m, 1 H), 4.08-4.14 (m, 1 H), 4.47-4.51 (m, 1 H), 5.07-5.14 (m, 3 H), 5.21 (d, J = 10.4 Hz, 1 H), 5.29 (d, J = 17.1 Hz, 1 H), 5.73-5.84 (m, 2 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 18.1, 19.4, 25.6, 25.8, 26.5, 27.0, 28.16, 28.2, 32.7, 33.0, 40.3, 41.9, 42.1, 71.9, 72.8, 73.0, 78.2, 169.0, 197. HRMS (FAB) calcd for $C_{31}H_{42}Co_2O_{11}SiH^+$ $[M+H]^+$: 737.1239, found 737.1240, Δ = -0.2 ppm.

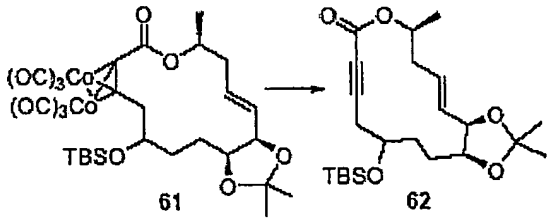


Macrolactone 61:

To a solution of **60** (339 mg, 0.460 mmol) in CH_2Cl_2 (80 mL) was added 2nd generation Grubbs catalyst (97 mg, 0.115 mol) in CH_2Cl_2 (15 mL) via cannula at r.t. The reaction mixture was stirred overnight and then the solvent was removed under reduced pressure vacuum and residue purified on preparative TLC (Whatman® Pk6F Silica Gel 60 Å 1000 μM) using Hexanes/EtOAc (10/1) as the eluant to afford *(2'R)*-**61** as a purple oil (123 mg, 38%). $[\alpha]_D^{25}$ -44.7 (*c* 0.25, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.08 (s, 3 H), 0.09 (s, 3 H), 0.91 (s, 9 H), 1.31 (d, J = 6.3 Hz, 3 H), 1.35 (s, 3 H), 1.45 (s, 3 H), 1.39-1.59 (m, 2 H), 1.65-1.72 (m, 1 H), 1.93-1.97 (m, 1 H), 2.22-2.31 (m, 1 H), 2.42 (dt, J = 12.9, 1.83 Hz, 1 H), 3.08 (dd, J = 16.0, 1.5 Hz, 1 H), 3.23 (dd, J = 16.0, 9.4 Hz, 1 H), 3.92-3.95 (m, 1 H), 4.01-4.06 (m, 1 H), 4.40 (dd, J = 9.4, 5.7 Hz, 1 H), 5.53 (ddd, J = 15.2, 10.9, 1.52 Hz, 1 H), 5.72 (ddd, J = 15.2, 10.7, 3.8 Hz, 1 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ -4.5, -4.4, 18.5, 21.2, 23.1, 26.1, 26.2, 28.8, 30.5, 41.0, 42.7, 71.6, 71.8, 79.3, 80.0, 81.3, 92.9, 108.1, 129.1, 132.6, 169.9, 198.8. HRMS (FAB)

calcd for $C_{29}H_{38}Co_2O_{11}SiH^+$ $[M+H]^+$: 709.0926, found: 709.0924, Δ = 0.2 ppm. *(2'R)*-**61** (136 mg, 42%). $[\alpha]_D^{25}$ -5.9 (*c* 0.27, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) δ 0.12 (s, 3 H), 0.15 (s, 3 H), 0.93 (s, 9 H), 1.32 (d, *J* = 6.3 Hz, 3 H), 1.35 (s, 3 H), 1.42 (s, 3 H), 1.11-1.54 (m, 2 H), 1.67 (m, 1 H), 1.88-1.93 (m, 1 H), 2.24-2.33 (m, 1 H), 2.41-2.45 (m, 1 H), 3.05 (dd, *J* = 14.9, 9.5 Hz, 1 H), 3.18 (dd, *J* = 14.9, 2.4 Hz, 1 H), 3.54-3.59 (m, 1 H), 4.03-4.09 (m, 1 H), 4.42 (dd, *J* = 9.5 Hz, 6.0 Hz, 1 H), 5.28 (m, 1 H), 5.53 (ddd, *J* = 15.2, 7.9, 4.0 Hz, 1 H), 5.77 (ddd, *J* = 15.2, 10.5, 3.5 Hz, 1 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ -4.3, -4.0, 18.4, 21.1, 25.8, 26.1, 26.2, 26.3, 26.9, 28.6, 32.4, 40.7, 44.6, 71.8, 73.8, 78.6, 79.8, 81.1, 93.3, 107.9, 128.6, 133.6, 169.6, 198.6. HRMS (FAB) calcd for $C_{29}H_{38}Co_2O_{11}SiH^+$ $[M+H]^+$: 709.0926, found: 709.0924, Δ = 0.2 ppm.

Note: the absolute configuration of 2'-OH was determined in compound **65**.

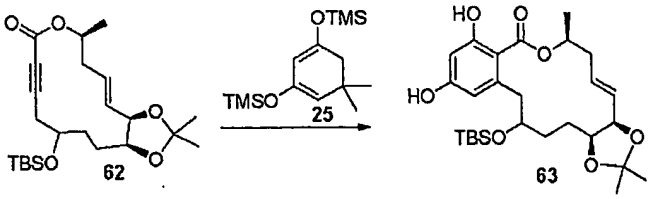


Macrolide 62:

To a solution of *(2'R)*-**61** (123 mg, 0.174 mmol) in acetone (10 mL) was added CAN (475 mg, 0.868 mmol) at -10 °C. After 20 min, the reaction mixture was filtered through neutral alumina and the solvent was removed under reduced pressure vacuum. The residue was purified on a silica gel column using petroleum ether/EtOAc (20/1) as the eluant to afford *(2'R)*-**62** as a colorless oil (69 mg, 94%). $[\alpha]_D^{25}$ -124.6 (*c* 0.17, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) δ -0.06, (s, 3 H), 0.00 (s, 3 H), 0.81 (s, 9 H), 1.24 (d, *J* = 6.2 Hz, 1 H), 1.33, (s, 3 H), 1.40 (s, 3 H), 1.59-1.81 (m, 4 H), 2.18-2.22 (m, 1 H), 2.28-2.31 (m, 1 H), 2.34-2.45 (m, 2 H), 3.90-3.93 (m, 1 H), 3.98-4.01 (m, 1 H), 4.33-4.36 (m, 1 H), 4.83-4.87 (m, 1 H), 5.42-5.53 (m, 2 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ -4.8,18.4, 20.6, 26.1, 26.3, 28.4, 28.8, 30.1, 36.5, 40.6, 70.0, 71.7, 78.9, 80.1, 88.5, 108.7, 129.7, 132.0, 153.8.

(2'S)-**62** was prepared by same procedure from *(2'S)*-**61**. *(2'S)*-**62**: (81 mg, 95%). $[\alpha]_D^{25}$ -173.3 (*c* 0.41, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) δ 0.00, (s, 3 H), 0.01 (s, 3 H), 0.81 (s, 9 H), 1.26 (d, *J* = 6.1 Hz, 3 H), 1.34 (s, 3 H), 1.41 (s, 3 H), 1.53-1.59

(m, 2 H), 1.77-1.81 (m, 2 H), 2.19-2.24 (m, 1 H), 2.24-2.26 (m, 1 H), 2.31-2.47 (m, 2 H), 3.80 (b, 1 H), 3.91-3.93 (m, 1 H), 4.34-4.40 (m, 1 H), 4.75-4.78 (m, 1 H), 4.82-4.86 (m, 1 H), 5.43-5.60 (m, 2 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ -5.0, -4.8, 17.9, 20.17, 20.24, 25.5, 25.6, 25.7, 25.9, 28.0, 28.2, 28.3, 28.4, 29.6, 36.0, 36.7, 40.0, 40.1, 69.5, 70.8, 71.2, 71.4, 78.5, 78.6, 79.4, 79.6, 78.8, 108.2, 129.2, 131.3, 153.0. HRMS (FAB) calcd for $C_{25}H_{42}O_5SiNa^+$ $[M+Na]^+$: 473.2699, found: 473.2700, Δ = 0.2 ppm.

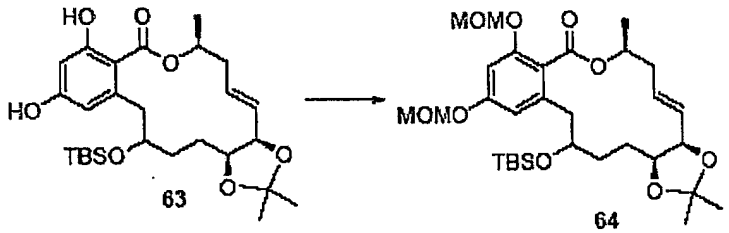


Resorcyclic macrolide 63:

Macrolide *(2'R)*-**62** (26 mg, 0.065 mmol) was transferred to a vial and 0.2 mL diene **25** was added. The vial was sealed and heated to 140 0 °C for 36 h. The crude mixture was purified on preparative TLC (Whatman® Pk6F Silica Gel 60 Å 1000 μM) using Hexanes/EtOAc (2/1) as the eluant to afford *(2'R)*-**63** as a colorless oil (23 mg, 74%). $[\alpha]_D^{25}$ -99.1 (*c* 0.08, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ -0.15, (s, 3 H), 0.09 (s, 3 H), 0.89 (s, 9 H), 1.36 (s, 3 H), 1.38 (s, *J* = 6.1 Hz, 1 H), 1.50 (s, 3 H), 1.25-1.39 (m, 4 H), 1.71-1.76 (m, 1 H), 2.54-2.57 (m, 2 H), 2.60-2.64 (m, 1 H), 3.63-3.68 (m, 2 H), 4.08-4.11 (m, 1 H), 4.48 (m, 1 H), 5.22-5.26 (m, 1 H), 5.54 (bs, 1 H), 5.70-5.75 (m, 2 H), 6.27 (d, *J* = 2.6 Hz, 1 H), 6.28 (d, *J* = 2.6 Hz, 1 H), 11.3 (s, 1 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ -4.5, -4.1, 0.4, 14.6, 18.4, 21.3, 21.5, 24.1, 25.9, 26.3, 28.6, 32.4, 40.1, 42.4, 70.0, 73.5, 73.7, 77.7, 79.7, 101.9, 106.6, 108.7, 111.6, 130.5, 131.6, 145.7, 160.4, 165.0, 172.1. HRMS (FAB) calcd for $C_{27}H_{42}O_7SiH^+$ $[M+H]^+$: 507.2778, found: 507.2777, Δ = 0.2 ppm.

(2'S)-**63** was prepared by same procedure from *(2'S)*-**62**. *(2'S)*-**63**: (81 mg, 84%). $[\alpha]_D^{25}$ -124.2 (*c* 0.42, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.00, (s, 3 H), 0.14 (s, 3 H), 0.99 (s, 9 H), 1.50 (s, 3 H), 1.58 (d, *J* = 6.1 Hz, 1 H), 1.40-1.67 (m, 2 H), 1.64 (s, 3 H), 1.87-1.92 (m, 2 H), 2.64-2.72 (m, 2 H), 3.09 (dd, *J* = 3.8 Hz, 2.5 Hz, 1 H), 3.45 (dd, *J* = 13.8, 7.8 Hz, 1 H), 4.02-4.04 (m, 1 H), 4.20-4.22 (m, 1 H), 4.70-4.74 (m, 1 H), 5.50-5.52 (m, 1 H), 5.76 (dd, *J* = 15.5, 8.1 Hz, 1 H), 5.93-5.95 (m, 2 H), 6.42 (m, 1 H), 6.48 (m, 1 H), 11.62 (s, 1 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ -4.3, -4.0, 14.5,

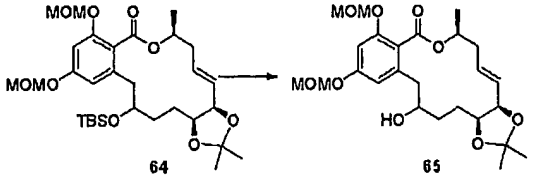
18.3, 18.4, 20.5, 21.5, 25.8, 26.2, 28.3, 28.4, 28.5, 33.1, 61.1, 72.7, 73.5, 79.1, 102.1, 107.1, 108.5, 112.7, 130.1, 130.5, 144.2, 160.7, 164.9, 171.7. LRMS (ESI) calcd for $C_{27}H_{42}O_7SiNa^+$ $[M+Na]^+$: 529.2, found: 529.1. LRMS (ESI) calcd for $C_{27}H_{42}O_7SiCl^-$ [M+Cl]: 541.3, found: 541.2.



MOM ether 64:

To a solution of *(2'R)*-**63** (23 mg, 0.045 mmol) in CH_2Cl_2 (0.5 mL) was added diethylpropylethylamine (0.08 mL, 0.450 mmol) and MOMCl (0.018 mL, 0.227 mmol). The reaction mixture was stirred for 10 h before quenched with saturated aqueous NH_4Cl solution, extracted with EtOAC (100 mL X 3). The organic layers were combined and dried with anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure vacuum. The residue was purified on preparative TLC (Whatman® Pk6F Silica Gel 60 Å 1000 μM) using Hexanes/EtOAc (2/1) as the eluant to afford *(2'R)*-**64** as a colorless oil (21 mg, 78%). $[\alpha]_D^{25}$ -2.86 (*c* 0.07, $CHCl_3$).). ^1H NMR (400 MHz, $CDCl_3$) δ 0.00, (s, 3 H), 0.10(s, 3 H), 0.93 (s, 9 H), 1.39 (s, 3 H), 1.39 (d, 3 H), 1.48 (s, 3 H), 1.42-1.48 (m, 1 H), 1.60-1.64 (m, 2 H), 1.72-1.77 (m, 1 H), 2.41-2.45 (m, 2 H), 2.66 (dd, *J* = 5.6, 4.6 Hz, 1 H), 2.89 (dd, *J* = 4.5, 1.6 Hz, 1 H), 3.47 (s, 3 H), 3.48 (s, 3 H), 3.97-3.98 (m, 1 H), 4.09-4.15 (m, 1 H), 4.73 (dd, *J* = 9.0, 6.0 Hz, 1 H), 5.13-5.19 (m, 4 H), 5.30-5.36 (m, 1 H), 5.57 (dd, *J* = 15.4, 9.1 Hz, 1 H), 5.70-5.77 (m, 1 H), 6.68 (d, *J* = 2.0 Hz, 1 H), 6.86 (d, *J* = 2.0 Hz, 1 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ -4.3, -4.2, 0.5, 18.4, 21.6, 24.0, 25.9, 26.3, 28.7, 30.1, 32.3, 40.1, 40.4, 56.5, 56.6, 71.4, 71.6, 80.3, 94.7, 94.9, 101.8, 108.5, 111.4, 119.8, 130.2, 132.4, 139.4, 155.3, 158.6, 168.4.

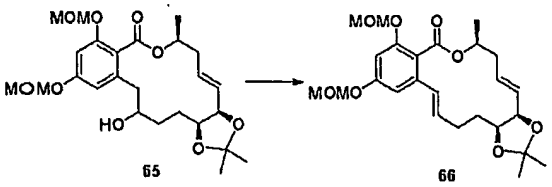
(2'S)-**64** was prepared by same procedure from *(2'S)*-**63** . *(2'S)*-**64**: (58 mg, 83%). $[\alpha]_D^{25}$ -16.2 (*c* 0.29, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) δ -0.17 (s, 3 H), -0.06 (s, 3 H), 0.81 (s, 9 H), 1.26 (s, 3 H), 1.33 (d, *J* = 6.1 Hz, 1 H), 1.38 (s, 3 H), 1.17-1.52 (m, 4 H), 2.30-2.36 (m, 2 H), 2.61 (dd, *J* = 14.2, 6.0 Hz, 1 H), 2.71 (dd, *J* = 14.2, 6.9 Hz, 1 H), 3.39 (s, 3 H), 3.40 (s, 3 H), 3.80-3.83 (m, 1 H), 4.10-4.14 (m, 1 H), 4.41 (dd, *J* = 8.9, 6.1 Hz, 1 H), 5.04-5.09 (m, 4 H), 5.20-5.23 (m, 1 H), 5.43 (dd, *J* = 15.3, 9.1 Hz, 1 H), 5.60-5.67 (m, 1 H), 6.46 (s, 1 H), 6.60 (s, 1 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ -4.2, -3.8, 18.4, 21.3, 25.8, 26.2, 26.25, 26.3, 28.6, 33.0, 39.8, 40.0, 42.0, 56.5, 56.6, 71.7, 74.5, 78.6, 79.9, 94.7, 94.9, 101.5, 108.3, 111.2, 119.8, 131.0, 131.4, 139.2, 155.8, 158.9, 168.5. HRMS (FAB) calcd for $C_{31}H_{50}O_9SiH^+$ $[M+H]^+$: 595.3302, found: 595.3304, Δ = -0.3 ppm.



Alcohol 65:

To a solution of *(2'R)*-**64** (21 mg, 0.035 mmol) in THF (1.4 mL) was added pyridine (0.6 mL) and HF-pyridine (30%, 0.3 mL). The reaction mixture was stirred for 10 h before quenched with MeOTf (2 mL) and stirred for 1 h. The solvent was removed under reduced pressure vacuum. The residue was purified on preparative TLC (Whatman® Pk6F Silica Gel 60 Å 1000 μM) using Hexanes/EtOAc (1/1) as the eluant to afford *(2'R)*-**65** as a colorless oil (12 mg, 78%). $[\alpha]_D^{25}$ -105.2 (*c* 0.06, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) δ -0.05 (s, 3 H), 0.05 (s, 3 H), 1.35 (s, 3 H), 1.38 (d, *J* = 6.2 Hz, 3 H), 1.46 (s, 3 H), 1.61-1.81 (m, 4 H), 2.40-2.46 (m, 2 H), 2.70 (dd, *J* = 14.1, 6.1 Hz, 1 H), 2.81 (dd, *J* = 14.1, 4.7 Hz, 1 H), 3.46 (s, 6 H), 3.89 (b, 1 H), 4.10-4.14 (m, 1 H), 4.56 (dd, *J* = 9.1, 6.1 Hz, 1 H), 5.13-5.17 (m, 4 H), 5.32-5.37 (m, 1 H), 5.59 (dd, *J* = 15.4, 9.2 Hz, 1 H), 5.70-5.75 (m, 1 H), 6.66 (d, *J* = 2.0 Hz, 1 H), 6.70 (d, *J* = 2.0 Hz, 1 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 21.5, 25.1, 25.7, 28.6, 30.1, 32.3, 40.0, 41.6, 56.6, 56.7, 70.5, 72.0, 80.1, 94.7, 94.9, 101.9, 108.2, 111.0, 119.7, 130.6, 132.7, 138.3, 155.8, 159.1, 168.4. HRMS (FAB) calcd for $C_{25}H_{36}O_9H^+$ $[M+H]^+$: 482.2438, found: 482.2437, Δ = 0.1 ppm.

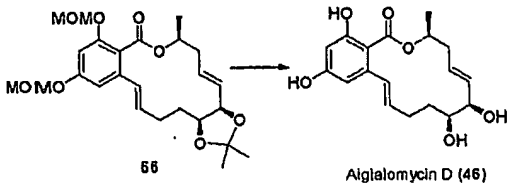
(2'S)-**65** was prepared by same procedure from *(2'S)*-**64**. *(2'S)*-**65**: (20 mg, 87%). $[\alpha]_D^{25}$ -32.0 (*c* 0.10, $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$) δ 1.18 (m, 2 H), 1.35 (s, 3 H), 1.37 (d, *J* = 6.2 Hz, 1 H), 1.42 (s, 3 H), 1.55-1.69 (m, 2 H), 1.98-2.06 (m, 1 H), 2.42-2.48 (m, 3 H), 2.78 (dd, *J* = 13.8, 2.4 Hz, 1 H), 3.41-3.50 (m, 6 H), 3.62-3.67 (m, 1 H), 4.19-4.25 (m, 1 H), 4.49 (dd, *J* = 9.4, 6.0 Hz, 1 H), 5.11-5.18 (m, 4 H), 5.36-5.42 (m, 1 H), 5.56 (dd, *J* = 15.4, 9.5 Hz, 1 H), 5.65-5.72 (m, 1 H), 6.57 (d, *J* = 2.0 Hz, 1 H), 6.66 (d, *J* = 2.0 Hz, 1 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 21.6, 25.8, 26.8, 28.6, 32.0, 39.8, 42.6, 56.5, 56.7, 72.3, 74.8, 80.3, 94.7, 94.9, 101.8, 108.6, 110.7, 108.6, 110.7, 119.5, 129.9, 132.8, 138.7, 155.9, 159.0, 168.7. HRMS (FAB) calcd for $C_{25}H_{36}O_9H^+$ $[M+H]^+$: 482.2438, found: 482.2437, Δ = 0.1 ppm.



Diene 66:

A solution of Martin's sulfurane dehydration agent (140 mg, 0.208) was added into a vial containing *(2'R)*-**65** (20 mg, 0.042) at 0 °C. The reaction mixture was warmed up to r.t. over 2 h and the crude was purified on preparative TLC (Whatman® Pk6F Silica Gel 60 Å 1000 μM) using Hexanes/EtOAc (1/1) as the eluant to afford **66** as a colorless oil (16 mg, 84%). $[\alpha]_D^{25}$ -123.8 (*c* 0.08, $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$) δ 1.35 (s, 3 H), 1.36 (d, *J* = 6.0 Hz, 3 H), 1.46 (s, 3 H), 1.49-1.55 (m, 1 H), 1.80-1.85 (m, 1 H), 2.07-2.11 (m, 1 H), 2.29-2.32 (m, 1 H), 2.45-2.55 (m, 2 H), 3.41-3.50 (m, 6 H), 4.16-4.21 (m, 1 H), 4.56 (dd, *J* = 9.5, 5.4 Hz, 1 H, 1 H), 5.10-5.20 (m, 4 H), 5.32-5.36 (m, 1 H), 5.59 (dd, *J* =15.5, 9.6 Hz, 1 H), 5.70-5.77 (m, 1 H), 5.15 (m, 1 H), 6.24 (d, *J* = 15.4 Hz, 1 H), 6.80 (d, J = 1.8 HZ, 1 H), 6.68 (d, J = 1.8 HZ, 1 H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 21.1, 25.8, 28.6, 28.7, 29.0, 39.5, 71.6, 80.1, 84.3, 94.6, 102.6, 104.8, 108.3, 117.9, 124.8, 128.4, 129.3, 131.9, 132.3, 136.8, 155.1, 158.9, 167.3. HRMS (FAB) calcd for $C_{25}H_{34}O_8H^+$ $[M+H]^+$: 463.2332, found: 463.2333, Δ = -0.2 ppm.

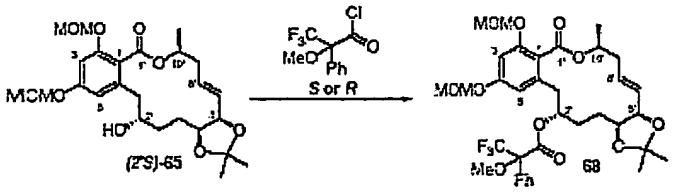
66 could also be obtained from *(2'S)*-**65** using same procedure (90%).



Aigialomycin D (46):

To a solution of **66** (16 mg, 0.035) in MeOH (1.5 mL) was added HCl (1 *N*, 1.5 mL) and stirred for 2 d. The reaction was quenched with saturated aqueous $NaHCO_3$ solution and extracted with EtOAc. The organic layers were combined and dried over anhydrous $MgSO_4$, filtered and concentrated under reduced vacuum. The crude was purified on preparative TLC (Whatman ® Pk6F Silica Gel 60 Å 1000 μM) using $MeOH/CH_2Cl_2$ (5%) as the eluant to afford **46** as a white solid (8 mg, 69%). Mp: 84.2-86.9 °C. $[\alpha]_D^{25}$ -18.0 (*c* 0.03, MeOH). IR (neat) 3346, 1643, 1607, 1311, 1261, 1166, 1017, 968. ^{1}H NMR (500 MHz, acetone-*d6*) δ 1.39 (d, *J* = 6.4 Hz, 3 H), 1.58-1.61 (m, 1 H), 2.14 (m, 1 H), 2.32-2.36 (m, 2 H), 2.43-2.46 (m, 1 H), 2.57 (ddd, *J* = 14.5, 7.3, 3.1 Hz, 1 H), 3.56 (br, 1 H), 3.64 (m, 1 H), 3.76 (br, 1 H), 4.35 (brd, *J* = 4.1 Hz, 1 H), 5.41-5.47 (m, 1 H), 5.69 (dd, *J* = 15.6, 5.1 Hz, 1 H), 5.87 (dddd, *J* = 15.6, 7.4, 7.4, 1.4 Hz, 1 H), 6.10 (ddd, *J* = 15.9, 5.5, 5.7 Hz, 1 H), 6.28 (d, *J* = 2.3 Hz, 1 H), 6.53 (d, *J* = 2.3 Hz, 1 H), 7.16 (d, *J* = 15.9 Hz, 1 H), 9.10 (bs, 1 H), 11.7 (s, 1 H). ^{13}C NMR (125 MHz, acetone-*d6*) δ 19.2, 28.1, 28.8, 38.1, 73.1, 73.4, 76.7, 102.6, 104.6, 107.9, 125.6, 130.8, 133.8, 135.9, 144.5, 163.2, 166.0, 172.3. LRMS (ESI) calcd for $C_{18}H_{22}O_6Na^+$ $[Na+H]^+$: 357.1, found: 357.3. LRMS (ESI) calcd for $C_{18}H_{21}O_6$ $[M-H]^-$: 333.1, found: 333.1. LRMS (ESI) calcd for $C_{18}H_{22}O_6Cl^-$-$[M+Cl]$: 369.1, found: 369.0. HRMS (TOF) calcd for $C_{18}H_{22}O_6Na^+$ $[M+Na]^+$: 357.1314, found: 357.1325, Δ = 3.1 ppm. All the physical data are consistent with the reported value (Isaka *et al. J. Org. Chem.***2002**, *67*, 1561-1566; incorporated herein by reference).

Determination of the stereochemistry at the **2'** position of **65**:



The stereochemistry of **2'** of *(2'S)*-**65** was determined according to the protocol reported by Ikuko Ohtani *et al.* (*Org. Chem.* **1991**, *56*, 1296-1298; incorporated herein by reference).

To a solution of XX (1.5 mg, 0.003 mmol) in CH_2Cl_2 (0.1 mL) was added triethylamine (0.007mL, 0.042 mmol), DMAP (0.1 mg, 0.001 mmol) and (*S*)-or (*R*)-methoxytrifluorophenyl acetic chloride (0.005 mL, 0.024 mmol). The reaction mixture was stirred overnight before loaded onto PTLC (250 μm) and eluted by Hex/EtOAc (4/1). The product (*R*) or (*S*) ester **68** was isolated as colorless oil (1.7 mg, 80%).

$\Delta\delta = \delta_S - \delta_R$.

$\Delta\delta_{3'} = \delta_{S5'} - \delta_{R5'} = -0.05$ ppm;

$\Delta\delta_{4'} = \delta_{S6'} - \delta_{R6'} = -0.13$ ppm;

$\Delta\delta_{5'} = \delta_{S7'} - \delta_{R7'} = -0.02$ ppm;

$\Delta\delta_{6'} = \delta_{S8'} - \delta_{R8'} = -0.05$ ppm;

$\Delta\delta_{1'} = \delta_{S1'} - \delta_{R1'} = 0.11$ ppm;

$\Delta\delta_5 = \delta_{S5} - \delta_{R5} = 0.05$ ppm;

$\Delta\delta_3 = \delta_{S3} - \delta_{R3} = 0.01$ ppm.

Therefore, the 2' position should have a *S* configuration as shown above.

Cell Culture. The human cancer line MCF7 was obtained from American Type Culture Collection (Manassas, Virginia, USA) and maintained in 1:1 mixture of DME:F12 supplemented with 10% heat-inactivated fetal bovine serum, 2 mM glutamine, 50 units/ml of penicillin and streptomycin, and incubated in a humidified 5% CO_2/ air atmosphere at 37° C. For the Alamar blue proliferation assay, 2 x 10^3 cells were plated in 96-well plates. Twenty four hours later, cells were treated with the compound for 72 or 120 hours. On day 3 or 5, Alamar Blue viability assay (AccuMed Westlake, OH) was performed as described elsewhere (White, M. J.; DiCaprio, M. J

Greenberg, D. A. *J. Neurosci. Methods* **1996**, *70*, 195-200; incorporated herein by reference). IC_{50}s were calculated as the doses of the compound required to inhibit cell growth by 50% of control values.

Immunoblotting. For immunoblotting, cells in culture were washed and harvested in PBS, and then lysed in NP-40 buffer (50mMTris, pH 7.5, 1% Nonidet P-40, 150 mM NaCl, 2.5 mM Na_3VO_4, 10 mM phenylmethylsulfonyl fluoride, and 10μM each leupeptin, aprotinin, and soybean trypsin inhibitor). Cell lysates were cleared by centrifugation at 14,000 x *g* for 10 minutes, and supernatants were collected as the experimental samples. Protein concentrations were determined using BCA reagent (Pierce Chemical Co., Rockford, IL). Lysates were added to sample buffer [0.3125 M Tris-HCl (pH 6.8), 10% SDS, 50% glycerol, and 77.5mg/ml DTT], and equal amounts of protein were resolved by 7% SDS-PAGE and transferred to nitrocellulose membranes. Blots were blocked in 5% nonfat milk in Tris-buffered saline [0.1% Tween 20, 10mM Tris (pH 7.4), and 150 mM NaCl] and subsequently probed with antibody (HER2 rabbit polyclonal IgG, Santa Cruz Biotechnology, Santa Cruz, CA; and PI3kinase P85, Upstate Cell Signaling Solutions, Lake Placid, NY). After incubation with horseradish peroxidase-conjugated secondary antibodies, proteins were visualized by chemiluminescence using the ECL detection reagents (Amersham Corp., Piscataway, NJ). To prepare lysate from xenograft tumors, tumor tissue was homogenized in 2% SDS lysis buffer for 30 s then processed as above.

Example 4-*In vitro* and *In vivo* Testing

Cell Culture Experimental:

The human cancer cell lines MCF7, BT474 and N417 were obtained from the American Type Culture Collection (Manassas, VA, USA) and maintained in a 1:1 mixture of DME:F12 supplemented with 2mM glutamine, 50 U/mL penicillin, 50 U/mL streptomycin and 5% heat inactivated fetal bovie serum (Gemini Bioproducts) and incubated at 37 °C in 5% CO_2.

Also, cytoxicity assay methods are described in Chou *et al. Proc. Natl. Acad. Sci. USA* 98:813-18, 2001; incorporated herein by reference.

Protein Assays:

Cells were grown to 60–70% confluence and exposed to drugs or DMSO vehicle for the indicated time periods. Lysates were prepared using 50mM Tris pH 7.4, 2% SDS and 10% glycerol lysis buffer. Protein concentration was determined using the BCA kit (Pierce Chemical Co.) according to the manufacturer's instructions. Clarified protein lysates (20–50 μg) were electrophoretically resolved on denaturing SDS-PAGE, transferred to ntirocellulose and probed with the following primary antibodies: anti-Her2 (C-18).

Antiproliferative Index:

Growth assays were performed by seeding 10000 cells (MCF7, BT474 or N417) per well in 6-welldishes and incubating for 24 h before drug treatment. Drugs or vehicle were administered as outlined for each experiment, and cells were incubated for the time periods depcited and then the number quantified by a Coulter counter.

Flow Cytometry:

Cell cycle distribution was assayed according to Nusse et al. with a Becton Dickinson fluorescence-activated cell sorter and analyzed by a Cell Cycle Multi-cycle system (Phoenix Flow System, San Diego, CA, USA).

***In vivo* activity:**

Although a variety of methods known in the art can be utilized, one exemplary method by which the *in vivo* activity of the inventive compounds is determined is by subcutaneously transplanting a desired tumor mass in mice. Drug treatment is then initiated when tumor mass reaches approximately 100 mm^3 after transplantation of the tumor mass. A suitable composition, comprising any one inventive compounds described above, including classes thereof, subclasses thereof, or species thereof, optionally further comprising a pharmaceutically acceptable carrier and optionally further comprising an additional therapeutic agenthas, is then administered to the mice, preferably in saline and also administered once a day at doses in the range of 0.001 mg/kg, to about 50 mg/kg, although it will be appreciated that other doses can also be

administered, as described herein (*e.g.*, 0.01 mg/kg to about 25 mg/kg of body weight, or 0.1 mg/kg to about 10 mg/kg of body weight), or, in some embodiments, at dosages in the range of about 50 mg/kg to about 100 mg/kg, or dosages below 0.001 mg/kg. Body weight and tumor size are then measured daily and changes in percent ratio to initial values are plotted. In cases where the transplanted tumor ulcerates, the weight loss exceeds 25-30% of control weight loss, the tumor weight reaches 10% of the body weight of the cancer-bearing mouse, or the cancer-bearing mouse is dying, the animal is sacrificed in accordance with NIH guidelines for animal welfare.

In addition, methods for studying the therapeutic effect of compounds *in vivo* in nude mice are described in Chou *et al. Proc. Natl. Acad. Sci. USA* 98:8113-18, 2001.

Results:

The methods for studying the cytotoxicity of the radicicol and cycloproparadicicol compounds *in vitro* for CCRF-CEM, CCRF-CEM/Vinblastine, and CCRF-CEM/Taxol cells are described in Chou *et al. Proc. Natl. Acad. Sci. USA* 98:8113-8118, 2001. The method of studying the therapeutical effec of these compounds *in vivo* is also described in Chou *et al. Proc. Natl. Acad. Sci. USA* 98:8113-8118, 2001.

The tested radicicol and cycloproparadicicol compounds suppressed xenograft tumor growth in nude mice about 60-88%. These effects are not dose dependent. All of the test compound that were tested *in vivo* are relatively non-toxic to normal tissues. The compounds do not show cross-resistance with vinblastine. Therefore, these compounds do not seem to be substrates for the multi-drug resistant (MDR) P-glycoprotein (P-gp). The compounds tested were also not found to be cross-resistant with Taxol. These compound may be particularly useful in conjunction with other anti-cancer agents. The radicicol and cycloproparadicicol compounds may increase the therapeutic effect of the combination without increasing the toxicity of the combination.